UNIVERSAL
LIBRARY

MONOGRAPHS ON MODERN CHEMISTRY

Editors

Professor James Kendall, M.A., D.Sc. (Edin.), F.R.S.

Professor John Read, M.A. (Camb.), B.Sc. (Lond.), Ph.D. (Zurich)

THE DONNAN EQUILIBRIA

THE
DONNAN EQUILIBRIA
AND THEIR APPLICATION TO CHEMICAL, PHYSIOLOGICAL AND TECHNICAL PROCESSES

BY

T. R. BOLAM

M.Sc. (Bristol), D.Sc. (Edinburgh)
Lecturer in Chemistry in the
University of Edinburgh

LONDON
G. BELL AND SONS, LTD.
1932

Printed in Great Britain by
NEILL & CO., LTD., EDINBURGH.

PREFACE

THE rapid growth of the literature dealing directly and indirectly with "Donnan equilibria" would seem to justify the presentation of a more detailed survey of the subject than has hitherto appeared. While the following account does not claim to be exhaustive, the endeavour has been made to include some reference to every development of importance.

Of necessity the arrangement of the subject-matter is somewhat arbitrary. The first chapter deals with the general theory and its more important implications. In the second, a description is given of fundamental investigations carried out on the more simple systems. Chapters III and IV are devoted to the discussion of biological and other equilibria, where conditions are less simple or the application of the theory is more speculative.

The theory of F. G. Donnan was worked out in the first instance for cases in which a definite membrane is present, but its progressive extension to cases where this is not so warrants the use of the title chosen.

It is hoped that the book will be of service not only to those who are primarily chemists, but also to biologists and others. While the development of the subject has proceeded so far mainly along purely scientific lines,

practical applications are not wanting and may be confidently expected to increase in number and importance.

I wish to express my thanks to Professor F. G. Donnan, F.R.S., and Professor J. P. Kendall, F.R.S., for their interest and many valuable suggestions; to Dr Mary R. Bolam and Miss Jean G. Ogilvie, B.Sc., for assistance with the manuscript and proofs; and to all who have afforded facilities for the production of the figures.

T. R. BOLAM.

EDINBURGH,
November 1931

CONTENTS

PAGE

INTRODUCTION 1

CHAPTER I

THEORETICAL OUTLINE 3

Simple kinetic theory—Simple thermodynamic theory —Membrane potentials—Osmotic pressure—Membrane hydrolysis—Condition for Donnan equilibrium.

CHAPTER II

SIMPLE CHEMICAL APPLICATIONS 39

Ferrocyanides—Colloidal dyes—Inorganic colloids—Proteins—Osmotic pressure of protein solutions —Carrageen extract—General theory—Neutralisation across a membrane—Sodium thymonucleate—Liquid membranes.

CHAPTER III

BIOLOGICAL AND TECHNICAL APPLICATIONS . . 80

Equilibria in the blood—Intra-ocular fluids—Other body fluids—Ionic nature of enzymes—Swelling of gels—Swelling of animal tissues—Manufacture of leather—Swelling of cotton hairs—Dyeing.

CHAPTER IV

PHYSICO-CHEMICAL APPLICATIONS 132

Determination of cataphoretic velocity — Determination of molecular weight by centrifuge — Viscosity of protein solutions — Distribution gradient within a gel—Electrokinetic potentials.

BIBLIOGRAPHY 145

INDEX 151

THE DONNAN EQUILIBRIA

INTRODUCTION

WILHELM OSTWALD (1) * appears to have been the first to direct attention to the interesting electrical and other effects which must arise in a system consisting of two solutions of electrolytes separated by a membrane which is permeable to most of the species of ions present, but is impermeable to at least one species. Ostwald's paper was published in 1890, but the subject remained uninvestigated until, some years later, Donnan and Harris (2), in the course of a research on the osmotic pressure of solutions of the dyestuff Congo-red, encountered membrane equilibria of the type in question.

The crucial observation of Donnan and Harris was made in the following manner. Sodium chloride was dissolved in an aqueous solution of Congo-red (the sodium salt of a complex sulphonic acid) and the mixture brought in contact with water through a membrane of parchment paper. The Congo-red was retained by the membrane, but the sodium chloride readily passed through. When the system was allowed to attain equilibrium and the chloride contents of the two solutions then determined, it was found that the concentration of sodium chloride was greater in the solution free from the dyestuff than in the other. Exactly the same final state was reached when the chloride was added to the water instead of to the Congo-red solution.

Donnan, (3), (4) realising the importance of the

* The numerals in brackets following names refer to publications the titles of which will be found in the Bibliography on pp. 145–149.

phenomenon, proceeded to develop a simple thermodynamic theory of such equilibria and to deduce the electrical and osmotic consequences of the unequal distribution of the ions. It was later realised that Willard Gibbs (5) had already worked out the general theory of heterogeneous systems in which at least one of the phases contains a substance unable to pass out of that phase, but able to combine with some other substance present, which is not subject to this restriction.

Since the appearance in 1911 of his fundamental paper, and especially during the last decade, the principles enunciated by Donnan have been repeatedly tested and their value established. In particular they have proved to be of the first importance in the study of physiological problems.

CHAPTER I

THEORETICAL OUTLINE

THE theoretical considerations detailed in the following lead to the general conclusion that the presence in any system of electrolytes of a species of ion, which is restrained in *any* way from diffusing to all parts of the system, will give rise to unequal distribution of every species of diffusible ion present. This particular state of *unequal ionic distribution* is the characteristic feature of "Donnan equilibria."

SIMPLE KINETIC THEORY

It will be useful in the first place to treat some important cases from the standpoint of simple kinetic theory,* assuming for convenience that the electrolytes concerned are completely ionised and that the presence of a membrane impermeable to one kind of ion provides the necessary restraint. We shall denote non-diffusible cations and anions by B^+ and A^- respectively. In the following discussion the non-diffusible ion is represented as being univalent; the treatment is the same when it is polyvalent.

(i) *Common Diffusible Ion.*—The Congo-red and sodium chloride system is an example of this case. We may represent the equilibrium condition by the following:

Na^+	Na^+
Cl^-	Cl^-
A^-	
(1)	(2)

* Wilson (6), Donnan (4), (8).

where the vertical line denotes a membrane. Since the system is in equilibrium, sodium chloride must be diffusing through the membrane at the same speed in both directions. Now an ion can pass through the membrane only if (**a**) an ion of the same sign simultaneously strikes the other side of the membrane, or (**b**) an ion of opposite sign strikes the same side at the same time. As has been pointed out by Ostwald (1), any other mechanism involves the separation of ionic charges, which, owing to the powerful electrostatic force set up, can proceed to only a very small extent. It is obvious that exchanges of the type (**a**) do not affect the final concentrations in any way. The condition for equilibrium is, therefore, that the frequency (N) with which sodium and chlorine ions strike the membrane is the same for both sides. N is proportional to the concentrations of both ions, *i.e.* $N_1 = k[Na^+]_1[Cl^-]_1$ and $N_2 = k[Na^+]_2[Cl^-]_2$, where the brackets denote molar concentrations. Hence at equilibrium ($N_1 = N_2$) we have

$$[Na^+]_1[Cl^-]_1 = [Na^+]_2[Cl^-]_2 \quad . \quad . \quad (1)$$

To fulfil the condition of electrical neutrality we have that $[Na^+]_2 = [Cl^-]_2$ and $[Na^+]_1 = [Cl^-]_1 + [A^-]$. From the first of these relations it follows that $[Na^+]_2[Cl^-]_2 = [Cl^-]_2^2$ and from the second that $[Na^+]_1 > [Cl^-]_1$. Hence $[Cl^-]_2 > [Cl^-]_1$, which means that the sodium chloride is more concentrated on the side of the membrane which is free from NaA.

It can readily be deduced from equation (1) that the inequality in the final sodium chloride concentrations may be surprisingly great under certain circumstances. To make the matter as simple as possible we will further assume that there are equal and constant volumes of liquid on the two sides of the membrane. We thus have:

Initial state				Final state			
c_1	Na^+	Na^+	c_2	c_1	A^-		
c_1	A^-	Cl^-	c_2	c_1+x	Na^+	Na^+	c_2-x
				x	Cl^-	Cl^-	c_2-x
	(1)	(2)		(1)		(2)	

The algebraic symbols represent molar concentrations; so that x mols of sodium chloride have passed from (2) to (1) when equilibrium is established. According to equation (1),

$$(c_1+x)x=(c_2-x)^2,$$

or

$$x=\frac{{c_2}^2}{c_1+2c_2},$$

from which it follows that

$$\frac{x}{c_2}=\frac{c_2}{c_1+2c_2}$$

and

$$\frac{c_2-x}{x}=\frac{c_1+c_2}{c_2} \quad . \quad . \quad . \quad (2)$$

Now $\frac{c_2-x}{x}$ is the distribution ratio of sodium chloride between (2) and (1). Hence the smaller the value of c_2 as compared with c_1, the less is the amount of chloride which passes into (1). If $c_2=\frac{c_1}{100}$, then $\frac{c_2-x}{x}=\frac{101}{1}$, *i.e.* almost the whole of the NaCl remains in (2), although the membrane is quite permeable to both ions of this salt. On the other hand, if c_2 is large compared with c_1, the chloride is divided practically equally between the two compartments.

(ii) *Common Non-Diffusible Ion.**—Let us suppose that

* Donnan and Garner (7).

the membrane separates two solutions containing initially only the salts NaA and KA respectively. Thus:

Initial state		Final state	
Na^+	K^+	Na^+	K^+
A^-	A^-	K^+	Na^+
		A^-	A^-
(1)	(2)	(1)	(2)

In this case the ionic exchanges will all be of the type (**a**) described above, but the exchange may take place in four different ways, namely,

($\mathbf{a}_1$)	Na^+	Na^+
($\mathbf{a}_2$)	K^+	K^+
($\mathbf{a}_3$)	Na^+	K^+
($\mathbf{a}_4$)	K^+	Na^+
	$\longrightarrow$	$\longleftarrow$
	(1)	(2)

($\mathbf{a}_1$) and ($\mathbf{a}_2$) obviously do not affect the final concentrations. The rate of exchange of type ($\mathbf{a}_3$) will be proportional to the concentration of sodium ions in (1) and also to the concentration of potassium ions in (2), *i.e.* $N_{a_3}=k[Na^+]_1[K^+]_2$. Similarly $N_{a_4}=k[Na^+]_2[K^+]_1$. At equilibrium these rates must be equal. Hence

$$[Na^+]_1[K^+]_2=[Na^+]_2[K^+]_1 \quad . \quad . \quad (3)$$

Assuming equal volumes of liquid in both compartments and representing the initial and final states as follows:—

Initial		Final	
c_1 Na^+	K^+ c_2	c_1-x Na^+	Na^+ x
c_1 A^-	A^- c_2	x K^+	K^+ c_2-x
		c_1 A^-	A^- c_2
(1)	(2)	(1)	(2)

we see from equation (3) that

$$(c_1 - x)(c_2 - x) = x^2$$

and

$$x = \frac{c_1 c_2}{c_1 + c_2}.$$

Hence

$$\frac{[Na^+]_1}{[K^+]_1} = \frac{[Na^+]_2}{[K^+]_2} = \frac{c_1 - x}{x} = \frac{c_1}{c_2}.$$

Suppose $c_2 = 10c_1$, then at equilibrium

$$\frac{c_2 - x}{x} = \frac{c_2}{c_1} = \frac{10}{1},$$

which means that 10/11 or 90·9 per cent. of the sodium ion originally present in (1) diffuses to (2), while only 1/11 or 9·1 per cent. of the potassium ion diffuses from (2) to (1). Only when $c_1 = c_2$ is it true for equilibrium that $[Na^+]_1 = [K^+]_1$ and $[Na^+]_2 = [K^+]_2$.

(iii) *Polyvalent Ions.*—So far we have dealt with monovalent diffusible ions. The case of ions with unequal valencies may now be considered. Suppose, for example, that we substitute the divalent calcium ion for the potassium ion in the last system, thus:

Initial		Final	
Na^+	Ca^{++}	Na^+	Ca^{++}
A^-	A^-	Ca^{++}	Na^+
		A^-	A^-
(1)	(2)	(1)	(2)

The effective ionic interchanges are of the nature of ($\mathbf{a}_3$) and ($\mathbf{a}_4$), but in order that a calcium ion may pass through the membrane *two* sodium ions must simultaneously strike the opposite side of the latter. The frequency (N_{a_3}) with which this happens on the left-hand side of the membrane is equal to $k[Na^+]_1[Na^+]_1[Ca^{++}]_2$

$=k[Na^+]_1^2[Ca^{++}]_2$. Similarly for the right-hand side we have $N_{a_4}=k[Na^+]_2^2[Ca^{++}]_1$. Since the final state depends only upon exchanges of Na^+ and Ca^{++}, it is reached when $N_{a_3}=N_{a_4}$, *i.e.* when

$$[Na^+]_1^2[Ca^{++}]_2=[Na^+]_2^2[Ca^{++}]_1,$$

or

$$\frac{[Ca^{++}]_1}{[Ca^{++}]_2}=\frac{[Na^+]_1^2}{[Na^+]_2^2}.$$

Again, consider the system

Initial		Final	
B^+	Ca^{++}	B^+	
Cl^-	Cl^-	Ca^{++}	Ca^{++}
		Cl^-	Cl^-
(1)	(2)	(1)	(2)

Here we are dealing with exchanges of the type (**b**); when a calcium ion strikes the membrane it passes through if two negative ions strike the membrane at the same time and pass through with it. The rate of transfer of Ca^{++} from (1) to (2) is therefore determined by the product $k[Ca^{++}]_1[Cl^-]_1^2$ and from (2) to (1) by $k[Ca^{++}]_2[Cl^-]_2^2$. Hence at equilibrium, when calcium must be diffusing in both directions at the same speed,

$$[Ca^{++}]_1[Cl^-]_1^2=[Ca^{++}]_2[Cl^-]_2^2 \quad . \quad (4)$$

(iv) *No Common Ion.*—Consider now the case in which we have initially BCl on one side (1) of the membrane and on the other an electrolyte with no ion in common, *e.g.* $NaNO_3$. It might appear that here at least the diffusible salt must distribute itself equally between (1) and (2). This, however, does not happen because *both* the types of interchange (**a**) and (**b**) are involved. Thus (neglecting exchanges of the same ion) while on the one

hand a sodium ion can pass through only in company with a negative ion, on the other, chloride and nitrate ions can interchange without the assistance of sodium. The effective combinations are thus:

(b_1)	Na^+ Cl^-		
(b_2)			Na^+ Cl^-
(b_3)	Na^+ NO_3^-		
(b_4)			Na^+ NO_3^-
(a_3)	Cl^-		NO_3^-
(a_4)	NO_3^-		Cl^-
	$\longrightarrow$		$\longleftarrow$
	(1)		(2)

At equilibrium

$$N_{b_1}=N_{b_2},\quad N_{b_3}=N_{b_4},\quad N_{a_3}=N_{a_4},$$

i.e.

$$[Na^+]_1[Cl^-]_1=[Na^+]_2[Cl^-]_2,$$
$$[Na^+]_1[NO_3^-]_1=[Na^+]_2[NO_3^-]_2,$$
$$[Cl^-]_1[NO_3^-]_2=[Cl^-]_2[NO_3^-]_1.$$

It will be seen that any one of these equations can be derived from the other two.

Assuming equal volumes of solution, we may represent the equilibrium state by

c_1	B^+		
x	Na^+	Na^+	c_2-x
c_1-z	Cl^-	Cl^-	z
y	NO_3^-	NO_3^-	c_2-y
(1)		(2)	

where c_1 and c_2 are the respective initial concentrations

of BCl and $NaNO_3$ and $x=y-z$. From the above equations we have

$$\frac{[Na^+]_2}{[Na^+]_1}=\frac{[Cl^-]_1}{[Cl^-]_2}=\frac{[NO_3^-]_1}{[NO_3^-]_2}=\lambda.$$

Hence

$$\lambda=\frac{[Na^+]_2+[Cl^-]_1+[NO_3^-]_1}{[Na^+]_1+[Cl^-]_2+[NO_3^-]_2}$$

$$=\frac{c_2-x+c_1-z+y}{x+z+c_2-y}=1+\frac{c_1}{c_2} \quad . \quad . \quad (5)$$

Suppose $c_1=10c_2$, then $\lambda=11$, and therefore

$$[Na^+]_2=11[Na^+]_1$$
$$[Cl^-]_1=11[Cl^-]_2$$
$$[NO_3^-]_1=11[NO_3^-]_2.$$

Thus when c_1 is large compared with c_2, most of the sodium ions are prevented from diffusing from (2) to (1), most of the nitrate ions pass into (1), and only a small fraction of the chlorine ions are transferred from (1) to (2). It is interesting to note that, if BCl is repeatedly dialysed against fresh sodium nitrate solution, the BCl is gradually converted into BNO_3.

(v) *Several Diffusible Electrolytes.*—On the basis of the considerations advanced in the foregoing, it can readily be shown that with any number of electrolytes in the system the product of the concentrations of any pair of univalent diffusible and oppositely charged ions will have the same value in both solutions at equilibrium, *e.g.* $[Na^+]_1[Cl^-]_1$ will always be equal to $[Na^+]_2[Cl^-]_2$, irrespective of the number and nature of the other ions present. A similar state of affairs exists in the case of polyvalent ions. Thus when calcium chloride is present the equilibrium is characterised by the relation $[Ca^{++}]_1[Cl^-]_1^2=[Ca^{++}]_2[Cl^-]_2^2$.

We shall examine in some detail the effect of a diffusible electrolyte with no common ion (say $NaNO_3$) on the distribution of HCl in the presence of BCl.

Initial state				Equilibrium			
c_1	B^+	H^+	c_2	c_1	B^+		
c_1	Cl^-	Cl^-	c_2	x	H^+	H^+	c_2-x
		Na^+	c_3	c_1+w	Cl^-	Cl^-	c_2-w
		NO_3^-	c_3	y	Na^+	Na^+	c_3-y
				z	NO_3^-	NO_3^-	c_3-z
(1)		(2)		(1)		(2)	

where $x+y=w+z$. Under equilibrium conditions

$$\frac{[H^+]_2}{[H^+]_1}=\frac{[Na^+]_2}{[Na^+]_1}=\frac{[Cl^-]_1}{[Cl^-]_2}=\frac{[NO_3^-]_1}{[NO_3^-]_2}=\lambda.$$

Hence

$$\lambda=\frac{c_2+c_3-(x+y)+c_1+(w+z)}{(x+y)+c_2+c_3-(w+z)}=1+\frac{c_1}{c_2+c_3} \quad . \quad (6)$$

When c_3 is large compared with c_1, λ approaches unity and the HCl is distributed approximately equally between (1) and (2), even though c_2 may be small compared with c_1, which in the absence of $NaNO_3$ would result in very unequal distribution of HCl (equation 2). An electrolyte without a common ion may therefore exert a very marked influence on the final state of a diffusible electrolyte which has an ion in common with the non-diffusible electrolyte.

Simple Thermodynamic Theory

In the following derivation * of the distribution equations, based on the second law of thermodynamics, it is assumed that the solutions are dilute, that the dissolved substances are completely ionised, and that

* Donnan (4), (8).

their ions act as ideal solutes. We shall again consider the cases discussed in the previous section, and it will be seen that the expressions obtained are identical with those derived by kinetic theory.

(i)

Equilibrium	
Na^+	Na^+
Cl^-	Cl^-
A^-	
(1)	(2)

As the system is in equilibrium, if a small virtual change is made reversibly at constant temperature and volume, the free energy will remain unchanged, *i.e.* no work will be done. The change here considered is the transfer of δn mols of Na^+ and δn mols of Cl^- from (2) to (1). Since equivalent quantities of positive and negative electricity have been transferred, electrical work terms cancel out and the work is

$$\delta n\, RT \ln \frac{[Na^+]_2}{[Na^+]_1} + \delta n\, RT \ln \frac{[Cl^-]_1}{[Cl^-]_2};\ *$$

and, since this is equal to zero, it follows that

$$\frac{[Na^+]_1}{[Na^+]_2} = \frac{[Cl^-]_2}{[Cl^-]_1}, \quad \text{or} \quad [Na^+]_1[Cl^-]_1 = [Na^+]_2[Cl^-]_2.$$

(ii)

Equilibrium	
Na^+	Na^+
K^+	K^+
A^-	A^-
(1)	(2)

The variation to be considered here is

δn mols Na^+ (1) ⟶ (2),
δn mols K^+ (2) ⟶ (1).

* "ln" = Napierian logarithm.

Hence the change in free energy is given by

$$\delta F = RT\left\{\delta n \ln \frac{[Na^+]_1}{[Na^+]_2} + \delta n \ln \frac{[K^+]_2}{[K^+]_1}\right\} = 0;$$

so that $\frac{[Na^+]_1}{[Na^+]_2} = \frac{[K^+]_2}{[K^+]_1}$, or $[Na^+]_1[K^+]_2 = [Na^+]_2[K^+]_1$.

(iii)

Equilibrium

Na^+	Na^+
Ca^{++}	Ca^{++}
A^-	A^-
(1)	(2)

Electrical equivalence will be preserved if δn mols Ca^{++} are transferred from (2) to (1) and *twice* that quantity of Na^+ from (1) to (2). We have therefore

$$\delta F = RT\left\{2\delta n \ln \frac{[Na^+]_1}{[Na^+]_2} + \delta n \ln \frac{[Ca^{++}]_2}{[Ca^{++}]_1}\right\} = 0.$$

Hence

$$\frac{[Ca^{++}]_1}{[Ca^{++}]_2} = \frac{[Na^+]_1{}^2}{[Na^+]_2{}^2}.$$

(iv)

Equilibrium

B^+	Na^+
Cl^-	Cl^-
Na^+	NO_3^-
NO_3^-	
(1)	(2)

For the infinitesimal change

δn mols Cl^- (1) $\longrightarrow$ (2),
δn mols NO_3^- (2) $\longrightarrow$ (1),

we have

$$\delta n\, RT \ln \frac{[Cl^-]_1}{[Cl^-]_2} + \delta n\, RT \ln \frac{[NO^-{}_3]_2}{[NO^-{}_3]_1} = 0.$$

Whence

$$\frac{[Cl^-]_1}{[Cl^-]_2} = \frac{[NO_3^-]_1}{[NO_3^-]_2}.$$

While the simple relations deduced above do not hold strictly in practice, they often provide useful approximations to the facts. More exact equations will be formulated at a later stage. For the present we shall deal with certain deductions from the simple theory.

Membrane Potentials *

An important consequence of the unequal distribution of ions on the two sides of a membrane is the production of a potential difference between the two solutions separated by the membrane. Such a potential difference is termed a "membrane potential." The calculation of its value in any particular case is illustrated by the following.

We will take the case of a diffusible common ion (Case i). Representing the equilibrium condition by

Na^+	Na^+
A^-	Cl^-
Cl^-	
(1)	(2)

let π_1 be the potential, for positive electricity, of (1), and π_2 that of (2). Let the extremely small quantity $\mathbf{F}\delta n$ of positive electricity be reversibly transferred at constant temperature from (2) to (1). This virtual variation from equilibrium involves the following terms:—

(*a*) The decrease in electrical free energy, represented by $\mathbf{F}\delta n(\pi_1 - \pi_2)$.

(*b*) The work obtained by the simultaneous transfer of

* Donnan (4), (8).

$p\delta n$ mols of Na^+ from (2) to (1) and of $q\delta n$ mols of Cl^- from (1) to (2), where $p+q$ must be equal to unity.* This work has the value

$$p\delta n\, RT \ln \frac{[Na^+]_2}{[Na^+]_1} + q\delta n\, RT \ln \frac{[Cl^-]_1}{[Cl^-]_2}.$$

Since the system is in equilibrium these quantities (*a*) and (*b*) are equal. Thus

$$\mathbf{F}\delta n(\pi_1 - \pi_2) = p\delta n\, RT \ln \frac{[Na^+]_2}{[Na^+]_1} + q\delta n\, .\, RT \ln \frac{[Cl^-]_1}{[Cl^-]_2};$$

but $$\frac{[Na^+]_2}{[Na^+]_1} = \frac{[Cl^-]_1}{[Cl^-]_2} = \lambda \text{ and } p+q=1,$$

and hence the membrane potential is

$$E_m = -(\pi_1 - \pi_2) = -\frac{RT}{\mathbf{F}} \ln \lambda = \frac{RT}{\mathbf{F}} \ln (1/\lambda) \quad . \quad (7)$$

We arrive at the same result if we consider the single variation

$$\delta n \text{ mols } Na^+ \qquad (2) \longrightarrow (1).$$

This would lead to the equilibrium $[Na^+]_2 = [Na^+]_1$, if no potential difference existed across the membrane. Since, however, it is known that the sodium ion concentrations are not equal, there must be a potential difference. Let ϵ denote the excess of positive potential of (1) over (2). Then the condition for equilibrium is that

$$\delta n\, RT \ln \frac{[Na^+]_2}{[Na^+]_1} - \mathbf{F}\delta n\epsilon = 0.$$

Hence $$\epsilon = \frac{RT}{\mathbf{F}} \ln \lambda.$$

It is instructive to deal with the question in another

* p and q represent the fraction of the total current carried by the respective ion, *i.e.* p and q are the transport numbers of the ions.

manner. Imagine that electrodes reversible to the sodium ion are placed in both (1) and (2). As the system is in equilibrium there can be no potential difference between these electrodes. If the membrane is now removed the system becomes an ordinary concentration cell, whose electromotive force (neglecting the liquid-liquid junction potential) is expressed by

$$E=\frac{RT}{\mathbf{F}}\ln\frac{[Na^+]_1}{[Na^+]_2}.$$

It follows that there must be a membrane potential difference equal to $\frac{RT}{\mathbf{F}}\ln\frac{[Na^+]_1}{[Na^+]_2}$, acting in opposition to that of the concentration cell.* Employing electrodes reversible to the chloride, we get similarly that the membrane potential must equal $\frac{RT}{\mathbf{F}}\ln\frac{[Cl^-]_2}{[Cl^-]_1}$.

This treatment, it will be seen, provides an alternative method of arriving at the fundamental distribution equation; for, since the membrane potential difference is independent of the nature of the electrodes, we have

$$\frac{[Na^+]_1}{[Na^+]_2}=E_m=\frac{[Cl^-]_2}{[Cl^-]_1}.$$

Both methods rest, of course, on the same thermodynamic basis.

According to equation (2),

$$\frac{[Cl^-]_2}{[Cl^-]_1}=1+\frac{c_1}{c_2},$$

* When $[Na^+]_1>[Na^+]_2$ the positive current due to the membrane potential difference tends to flow from (1) to (2), whereas that due to the electrode potentials tends to flow from (2) to (1), through the cell.

where c_1 and c_2 are the initial concentrations of NaA and NaCl respectively. Therefore

$$E_m = 2{\cdot}303 \frac{RT}{\mathbf{F}} \log \left(1+\frac{c_1}{c_2}\right)^*$$

which becomes $0{\cdot}058 \log \left(1+\frac{c_1}{c_2}\right)$ volts at 18°. The Table I shows how the potential varies with the ratio c_1/c_2.

TABLE I

c_1/c_2	E_m† (18°)
1	+0·017
10	+0·060
100	+0·116
1000	+0·174

If c_1 is small compared with c_2, the potential difference approximates to zero, which is to be expected, since in the limiting case, where NaA is absent altogether, the NaCl will naturally distribute itself so that the concentration is the same on both sides of the membrane.

Equation (7) is still valid when ions of differing valency are present in the system. Applying the reasoning given above to the general case of the diffusible chloride possessing the ion M^{n+}, of valency n, the potential is found to be

$$E_m = \frac{RT}{n\mathbf{F}} \ln \frac{[M^{n+}]_1}{[M^{n+}]_2}.$$

* "log" = common logarithm.

† The positive sign shows that solution (2) is at the higher potential.

But from equation (4)

$$\frac{[M^{n+}]_1}{[M^{n+}]_2}=\frac{[Cl^-]_2^n}{[Cl^-]_1^n}=(1/\lambda)^n,$$

hence

$$E_m=\frac{RT}{n\mathbf{F}}\ln(1/\lambda)^n=\frac{RT}{\mathbf{F}}\ln(1/\lambda).$$

However complicated the system, the value of E_m will be given by $\frac{RT}{\mathbf{F}}\ln(1/\lambda)$, where λ is the ratio (at equilibrium) $\frac{\text{conc. in (2)}}{\text{conc. in (1)}}$ for any species of ion. Although the addition of any diffusible ionogen to a system already in equilibrium must produce a change in the potential difference, by displacing the equilibrium, when this is again established all the ions present are producing the same potential, whatever their number or valencies. We may, for example, consider the system

Initial state				Final state			
Na^+ A^-	Na^+ Cl^-			Na^+ K^+		Na^+ K^+	
	K^+ NO_3^-			Cl^- NO_3^-	A^-	Cl^- NO_3^-	
(1)	(2)			(1)		(2)	

At equilibrium

$$\frac{[Na^+]_2}{[Na^+]_1}=\frac{[K^+]_2}{[K^+]_1}=\frac{[Cl^-]_1}{[Cl^-]_2}=\frac{[NO_3^-]_1}{[NO_3^-]_2}=\lambda.$$

The value of the membrane potential is equal to the electromotive force of the concentration cell; Na (metal), $[Na^+]_1$, $[Na^+]_2$, Na (metal), *i.e.* to $\frac{RT}{\mathbf{F}}\ln\frac{[Na^+]_1}{[Na^+]_2}$.

Hence $$E_m=\frac{RT}{\mathbf{F}}\ln(1/\lambda).$$

Approaching the question in the alternative fashion,

we equate $\mathbf{F}\delta n$, the decrease in electrical free energy which corresponds to the transfer of

$$\begin{array}{ll} p\delta n \text{ mols } Na^+ & (2) \longrightarrow (1), \\ q\delta n \text{ mols } K^+ & (2) \longrightarrow (1), \\ t\delta n \text{ mols } Cl^- & (1) \longrightarrow (2), \\ s\delta n \text{ mols } NO_3^- & (1) \longrightarrow (2), \end{array}$$

where $$p+q+t+s=1,$$

with the osmotic work gained by this operation. That is to say we have

$$\begin{aligned} \mathbf{F}\delta n(\pi_1-\pi_2) &= \delta n\, RT(p \ln [Na^+]_2/[Na^+]_1 + q \ln [K^+]_2/[K^+]_1 \\ &\quad + t \ln [Cl^-]_1/[Cl^-]_2 + s \ln [NO_3^-]_1/[NO_3^-]_2) \\ &= \delta n\, RT \ln \lambda. \end{aligned}$$

Hence $$E_m = \frac{RT}{\mathbf{F}} \ln (1/\lambda).$$

Osmotic Pressure *

Another effect of the unequal distribution of the diffusible electrolyte is the production of an osmotic pressure acting in opposition to that of the non-diffusible electrolyte. Reverting to the equilibrium system

c_1+x	Na^+	Na^+	c_2-x
x	Cl^-	Cl^-	c_2-x
c_1	A^-		
	(1)	(2)	

where equal volumes of liquid are assumed, we have the true osmotic pressure of NaA given by

$$P_0 = 2RTc_1$$

and the counter osmotic pressure due to the unequal distribution of the sodium chloride by

$$P = 2RT(c_2-x) - 2RTx.$$

* Donnan (4), (8).

Hence the expression for the *observed* osmotic pressure is

$$P_1 = P_0 - P = 2RTc_1 - 2RT(c_2 - 2x) \quad . \quad (8)$$
$$= 2RT(c_1 - c_2 + 2x)$$

and since

$$x = \frac{c_2^2}{c_1 + 2c_2} \text{ (p. 5)}, \quad \frac{P_1}{P_0} = \frac{c_1 + c_2}{c_1 + 2c_2} \quad . \quad (9)$$

Table II shows how P_1/P_0 varies with the values of c_1 and c_2.

TABLE II

c_2/c_1	P_1/P_0
0·1	0·92
1·0	0·67
2·0	0·60
10·0	0·52

Thus if c_2 is large compared with c_1 the apparent osmotic pressure will be approximately equal to RTc_1 and the ionisation of NaA appear to be completely repressed, even though c_1 and c_2 may actually be small. Suppose A^- were an anion of colloidal dimensions with a molecular weight of 5000, then c_1 would be about 0·002 molar for a 1 per cent. solution. With $c_2 = 0{\cdot}02$ molar the apparent osmotic pressure of NaA would be only 4 per cent. greater than the value corresponding to zero ionisation of the non-diffusible electrolyte.

It is obvious that neglect of the Donnan equilibrium may introduce very serious errors into the determination of the molecular weight of a non-diffusible ion by measurements of the osmotic pressure. For example, if in the above case we ignore the unequal distribution of the diffusible electrolyte and take the observed pressure as that of NaA, the apparent molecular weight

of A is twice the true value. Actually the observed pressure is due to the non-diffusible ion alone.

Membrane Hydrolysis *

The Donnan equilibrium is of importance in connection with the phenomenon of "membrane hydrolysis." This process occurs when we have a solution of a salt of the type NaA or BCl separated from pure water by a membrane. Taking the case of NaA, we have initially:

Na^+ A^-	Water
(1)	(2)

The sodium ion strives to pass from (1) to (2), and does so when an equivalent amount of OH^- ion, furnished by the water in (1), can diffuse with it. Finally we get the equilibrium state

Na^+ A^- H^+	Na^+ OH^-
(1)	(2)

in which (1) is acid and (2) alkaline.

We obtain the equation for the distribution of the NaOH by applying the principle of virtual work as previously. For the transfer of

$$\delta n \text{ mols } Na^+ \quad (1) \longrightarrow (2),$$
$$\delta n \text{ mols } OH^- \quad (1) \longrightarrow (2),$$

we have

$$\delta \mathbf{F} = \delta n\, RT \ln \frac{[Na^+]_1}{[Na^+]_2} + \delta n\, RT \ln \frac{[OH^-]_1}{[OH^-]_2},$$

from which follows

$$\frac{[Na^+]_1}{[Na^+]_2} = \frac{[OH^-]_2}{[OH^-]_1} \quad . \quad . \quad . \quad (10)$$

* Donnan (4).

To simplify matters as much as possible we shall assume that HA is a strong acid, that there are equal volumes of solution in (1) and (2), and that $[H^+]_1$ and $[OH^-]_2$ are large in comparison with the concentrations in pure water. Under these conditions we have

Initial state			Final state		
c_1 Na^+	H_2O		c_1-x Na^+	Na^+ x
c_1 A^-			x H^+	OH^- x
			c_1 A^-	
(1)	(2)		(1)	(2)

From equation (10)

$$\frac{c_1-x}{x}=\frac{x}{[OH^-]_1}.$$

Also

$$x[OH^-]_1=k_w \text{ (the ionic product of water).}$$

Hence

$$x^3=k_w(c_1-x).$$

When x is small compared with c_1, this expression reduces to

$$x=\sqrt[3]{k_w c_1}.$$

Since the value of k_w at ordinary temperatures is about 10^{-14}, it is evident that $100x/c_1$, the percentage hydrolysis of NaA, must have very small values under the prescribed circumstances. As is shown by the following table, x increases slowly relatively to the increase in c_1:—

TABLE III

c_1	x	$100x/c_1$
0·01	$5\cdot10^{-6}$	0·05
0·1	$1\cdot10^{-5}$	0·01
1·0	$2\cdot10^{-5}$	0·002

The hydrolysis is favoured by increasing the volume of liquid in (2). Suppose the volume in (2) is made v times as great as that in (1), then the equilibrium state will be represented by

c_1-x	Na^+		Na^+	x/v
x	H^-		OH^-	x/v
c_1	A^-			
(1)			(2)	

which leads to

$$x=\sqrt[3]{k_w v^2 c_1}$$

when x is small compared with c_1. Thus if $v=100$ and $c_1=0{\cdot}1$, then $100/c_1$ will be of the order of magnitude 10^{-1}.*

Naturally the hydrolysis is greater if HA is insoluble or weak.

General Theory †

Activities.—The simple thermodynamic treatment given in preceding sections applies to dilute ideal solutions, where the change in free energy resulting from the transfer of δn mols of an ion from a solution (2) in which its molar concentration is c_2 to one (1) in which its molar concentration is c_1, is given by $\delta F=\delta n\, RT \ln c_2/c_1$. In non-ideal solutions this does not hold, but we may preserve the form of the expression and write

$$\delta F=\delta n\, RT \ln \frac{\text{activity of ion in (2)}}{\text{activity of ion in (1)}}.$$

More general equations for the various membrane equilibria are therefore obtained by substituting activities for concentrations. For example, the distribution of the

* This treatment for unequal volumes of liquid may be applied to the systems discussed earlier.

† Donnan (9), Hückel (10).

sodium chloride in Case (i) (see p. 4) is represented more correctly by

$$(a_{Na+})_1(a_{Cl-})_1=(a_{Na+})_2(a_{Cl-})_2 \quad (a=\text{activity}).$$

For convenience the assumption was made that the electrolytes were completely ionised, but the equations still hold if incomplete dissociation be postulated. Since, however, we possess no certain means of determining the actual ionic concentrations in solutions of strong electrolytes, it is customary to take the activity of an ion as referring to the stoichiometrical or total concentration m of the radicle which forms the ion. That is to say, we put $a_i=f_i m_i$, where f_i is the activity coefficient of the ion. In other words, f_i expresses the actual behaviour of the ion without assumptions as to the particular causes of that behaviour. We thus regard any formation of un-ionised molecules as a departure from ideal behaviour on the part of the ion. Adopting this convention, the equation of distribution becomes

$$(f_{Na}m_{Na})_1(f_{Cl}m_{Cl})_1=(f_{Na}m_{Na})_2(f_{Cl}m_{Cl})_2.$$

At sufficiently high dilution, when the ions are quite independent (no inter-ionic action or formation of un-ionised molecules), the equation reduces to

$$(m_{Na})_1(m_{Cl})_1=(m_{Na})_2(m_{Cl})_2 \quad (cf.\ \text{equation 1}).$$

The distribution equation can be put in a form still more suitable for application to actual data by introducing the geometric mean of the activities of the ions. Thus for a given solution of sodium chloride we have:

$$\text{Mean activity of ions}=a=(a_{Na}\,.\,a_{Cl})^{\frac{1}{2}}$$
$$=(f_{Na}m_{Na}\,.\,f_{Cl}m_{Cl})^{\frac{1}{2}}=(f_{Na}f_{Cl}\,.\,m_{Na}m_{Cl})^{\frac{1}{2}}=f_{NaCl}m_{NaCl}=fm,$$

where f and m are respectively the mean activity

coefficient and mean molarity of the ions. Hence for equilibrium we have

$$f_1 m_1 = f_2 m_2 \quad . \quad . \quad . \quad (11)$$

Osmosis.—At equilibrium the partial free energy of the water must be the same on the two sides of the membrane, *i.e.*

$$(\overline{F}_{H_2O})_1 = (\overline{F}_{H_2O})_2.$$

In general the establishment of this condition entails osmosis of the water through the membrane, and it becomes necessary to consider how far the ionic equilibrium is affected by the difference of pressure so produced. Hückel (10) has shown how the formulæ obtained by the simple theory are to be modified. He deals with the equilibrium

$c_1 + c_3$	Na^+	Na^+	c_2
c_1	Cl^-	Cl^-	c_2
c_3	A^-		
(1)		(2)	

where c_1, c_2, and c_3 are molar concentrations. The formula deduced is

$$[2(c_1 + c_3) - 2c_2]\frac{v_1 + v_2}{v_0} = 2 \ln c_2 - \ln c_1(c_1 + c_3),$$

where v_1, v_2, and v_3 are the respective molecular volumes of Na^+, Cl^-, and water. In general $v_1 + v_2$ does not differ very greatly from v_0. Hence in sufficiently dilute solutions, where c_1, c_2, and c_3 are small compared with unity, the above equation may be reduced to

$$2 \ln c_2 - \ln c_1(c_1 + c_3) = 0,$$

or

$$c_2^2 = c_1(c_1 + c_3),$$

which is Donnan's equation.

Kameyama (11) has dealt with the problem in the following manner. If $\overline{V}_K$ is the partial molar volume of the potassium ion in a solution of potassium chloride and P is the pressure, then

$$\overline{V}_K=\left(\frac{\partial \overline{F}_K}{\partial P}\right)_T=RT\left(\frac{\partial \ln a_K}{\partial P}\right)_T=RT\left(\frac{\partial \ln f_K}{\partial P}\right)_T,$$

therefore

$$\frac{1}{f_K}\left(\frac{\partial f_K}{\partial P}\right)_T=\frac{\overline{V}_K}{RT}.$$

Similarly

$$\frac{1}{f_{Cl}}\left(\frac{\partial f_{Cl}}{\partial P}\right)_T=\frac{\overline{V}_{Cl}}{RT}.$$

Hence

$$\frac{1}{f_{KCl}}\left(\frac{\partial f_{KCl}}{\partial P}\right)_T=\frac{\overline{V}_K+\overline{V}_{Cl}}{2RT}=\frac{\overline{V}_{KCl}}{2RT}.$$

If the pressure change is moderate we may write

$$\frac{\Delta f_{KCl}}{f_{KCl}}=\frac{\overline{V}_{KCl}}{2RT}.\ \Delta P.$$

Kameyama gives the following data for aqueous solutions of potassium chloride at 25°:—

Molarity .	0·1668	0·2740	0·3385	0·6840	0·9472
$\overline{V}$ (c.c.) .	+28·0	28·5	28·7	29·4	29·7

Assuming that $\overline{V}=28{\cdot}0$, we get

$$\frac{\Delta f_{KCl}}{f_{KCl}}=6\times10^{-4}\Delta P,$$

where P is expressed in atmospheres. This means that a

difference in osmotic pressure of one atmosphere causes an increase in f_{KCl} of only 0·06 per cent. Hence in the case of dilute solutions of potassium chloride the effect due to osmosis may be neglected.

Gibbs's Equation.—Adair (12) and Donnan (13) have pointed out that the theory of membrane equilibria lies implicit in the work of Willard Gibbs (5). The following quotation is from Donnan's paper: "Let us suppose that the diaphragm is permeable to a linearly correlated group of components, *i.e.* a number of components which can pass through the diaphragm only together and in amounts which stand to each other in a constant proportion. We may express this condition by the equations $\left(\frac{dm_1}{a_1}\right)_A = \left(\frac{dm_2}{a_2}\right)_A = \text{etc.}$, where a_1, a_2, etc., are constant positive quantities and A refers to the liquid phase on one side of the diaphragm. A similar series of equations holds for the B-phase, the quantities a_1, a_2, etc., being taken negatively. The general equation of equilibrium

$$(\Sigma \mu dm)_A + (\Sigma \mu dm)_B = 0$$

then gives at once the relation obtained by Gibbs,

$$(\Sigma a\mu)_A = (\Sigma a\mu)_B,$$

where the signs of summation apply to the correlated group of components."

In the case of, say, sodium chloride, there is linear correlation of the two ions due to the operation of electrostatic forces. The Gibbs equation applied to the diffusion of equivalent masses of Na and Cl gives

$$(\mu_{Na} + \mu_{Cl})_A = (\mu_{Na} + \mu_{Cl})_B,$$

where the thermodynamic potentials (μ) refer to these

equivalent masses. In terms of activities this relation becomes

$$(\ln a_{Na} + \ln a_{Cl})_A = (\ln a_{Na} + \ln a_{Cl})_B,$$

i.e.

$$(a_{Na} \cdot a_{Cl})_A = (a_{Na} \cdot a_{Cl})_B.$$

Neutralisation across a Membrane

Hitchcock (14) has applied the Donnan theory to the case of a non-diffusible base, acid or ampholyte of known ionisation constant. The following is taken largely from his paper and deals with the addition of a strong acid or base to systems containing non-diffusible electrolytes of the above type. In his treatment Hitchcock uses concentrations instead of activities, with the understanding that the equations can be exact only for solutions so dilute that these quantities become identical. Complete ionisation for strong electrolytes is assumed.

The equilibrium state of the systems under consideration is represented by the diagram given below, the valence of the cation (M) of the added base being represented by q and that of the anion (N) of the added acid by p. The non-diffusible molecules are denoted by R, R^+, and R^-.

$c-w-z$	R		
z	R^+		
w	R^-		
y	H^+	H^+	x
v	OH^-	OH^-	u
n/q	M^{q+}	M^{q+}	m/q
$1/p(y+z+n-w-v)$	N^{p-}	N^{p-}	$1/p(x+m-u)$

According to Donnan's equation for the ion ratio,

$$\lambda = \frac{x}{y} = \frac{v}{u} = \sqrt[q]{\frac{m}{n}} = \sqrt[p]{\frac{y+z+n-w-v}{x+m-u}} \quad . \quad (12)$$

The constancy of the ion product for water gives

$$k_w = xu = yv.$$

Applying the law of mass action to the ionisation of the non-diffusible electrolyte, we have

$$k_a = \frac{yw}{c-w-z} \quad . \quad . \quad . \quad (13)$$

$$k_b = \frac{vz}{c-w-z} \quad . \quad . \quad . \quad (14)$$

The condition of electrical neutrality in each solution is implied by the values for the concentrations of the ion A.

I. *Addition of a Monobasic Acid to a Non-Diffusible Base.*—In this case w, k_a, m, and n are all equal to zero, while $p=1$. Equation (12) reduces to

$$\lambda = \frac{x}{y} = \frac{v}{u} = \frac{y+z-v}{x-u} = \frac{y+z}{x} \quad . \quad . \quad (15)$$

Putting $x=\lambda y$, we therefore have

$$\lambda = \sqrt{1+\frac{z}{y}} \quad . \quad . \quad . \quad (16)$$

By substitution of k_w/y for v and K for k_w/k_b, equation (14) becomes

$$\frac{z}{c-z} = \frac{y}{K}, \quad \text{or} \quad z = \frac{cy}{K+y} \quad . \quad . \quad (17)$$

Hence, from equations (16) and (17),

$$\lambda = \sqrt{1+\frac{c}{K+y}} \quad . \quad . \quad . \quad (18)$$

This equation shows that as the concentration of hydrogen ion is increased the ion ratio λ must decrease, approaching the limit 1. This is made clear by the following. From equation (18) we see that $\frac{d\lambda}{dy}$ must

always be negative. Since $\lambda=\frac{x}{y}$, $\frac{d\lambda}{dy}=\frac{1}{y}\cdot\frac{dx}{dy}-\frac{x}{y^2}$, and since $\frac{d\lambda}{dy}$ is negative, $\frac{x}{y}>\frac{dx}{dy}$. Also $\frac{d\lambda}{dx}=\frac{1}{y}-\frac{x}{y^2}\cdot\frac{dy}{dx}$. Now if $\frac{d\lambda}{dx}$ is positive or zero, then $\frac{1}{y}\geqq\frac{x}{y^2}\cdot\frac{dy}{dx}$, and $\frac{dx}{dy}\geqq\frac{x}{y}$. But it has just been shown that this does not hold, and hence $\frac{d\lambda}{dx}$ must be negative. Finally $\frac{dy}{dx}$ and $\frac{dx}{dy}$ must always be positive since they are quotients of the two negative derivatives $\frac{d\lambda}{dx}$ and $\frac{d\lambda}{dy}$.

When the acid and base are present in equivalent amounts and the volumes of the two solutions are kept equal

$$x+y+z=c+u+v.$$

Since the solution will be acid, v is negligible in comparison with y. Also u may be neglected, since $\lambda>1$ and thus u is even smaller than v. The equation therefore approximates to

$$c=x+y+z \quad . \quad . \quad . \quad (19)$$

From equations (17) and (19)

$$c=\frac{(K+y)z}{y}=x+y+z,$$

and

$$z=\frac{y(x+y)}{K}.$$

From equation (15)

$$z=\frac{x^2}{y}-y=\frac{(x-y)(x+y)}{y}.$$

By elimination of z we therefore have

$$\frac{y}{K}=\frac{x-y}{y}=\lambda-1.$$

That is to say, equation (18) assumes the simple form

$$\lambda=1+\frac{y}{K}.$$

Equation (18) predicts that in the case of a non-amphoteric base, which is appreciably ionised in pure water, the membrane potential will have a high value and can only be decreased by the addition of acid. Donnan (8) has also dealt with the case of a non-amphoteric base, but arrives at the conclusion that the addition of acid causes initially an increase in the membrane potential. This is due to the assumption that the ionisation of the base can be neglected. Donnan's treatment is strictly correct for the case of a non-diffusible substance which does not ionise at all in pure water, but which forms ions by combination with the hydrogen ions of an added acid.

The osmotic pressure difference * in the general case of a system containing a non-diffusible base and a strong acid should be given by

$$P_1=RTe=RT(c+2y+z-2x).$$

It will be seen that $e-c$ is the difference between the total concentrations of the diffusible ions in the two solutions. Since the changes in osmotic pressure with pH are dependent upon $e-c$, the nature of these changes may be deduced from the sign of the derivative.

From equation (15)

$$y+z=\frac{x^2}{y} \quad \text{and} \quad \lambda=\frac{x}{y}.$$

* See also Donnan (8).

Hence

$$e-c=y+z-2x+y=y(\lambda-1)^2 \quad . \quad (20)$$

and

$$\frac{d(e-c)}{dy}=\frac{de}{dy}=(\lambda-1)\left(\lambda-1+2y\frac{d\lambda}{dy}\right).$$

From equation (18) $\lambda>1$ (except that, when y becomes infinite, $\lambda=1$); hence $\lambda-1$ is positive. Hence, since $\frac{d\lambda}{dy}$ is negative, the value of $\frac{de}{dy}$ will be positive if $\lambda-1>-2y\frac{d\lambda}{dy}$, negative if $\lambda-1<-2y\frac{d\lambda}{dy}$, and zero if $\lambda-1=-2y\frac{d\lambda}{dy}$.

Differentiating equation (18), we have

$$\frac{d\lambda}{dy}=-\frac{c}{2\lambda(K+y)^2}.$$

Therefore $\frac{de}{dy}$ is positive when $\lambda-1>\frac{cy}{\lambda(K+y)^2}$. But since, from equation (18), $\frac{cy}{(K+y)^2}=\frac{(\lambda^2-1)y}{K+y}$, this condition becomes

$$(\lambda-1)\lambda>\frac{(\lambda^2-1)y}{K+y},\ \lambda>\frac{(\lambda+1)y}{K+y},\ \frac{K+y}{y}>\frac{\lambda+1}{\lambda},\ \frac{K}{y}>\frac{1}{\lambda},$$

or $\lambda>\frac{y}{K}$. Similarly $\frac{de}{dy}$ is negative when $\lambda<\frac{y}{K}$ and zero when $\lambda=\frac{y}{K}$. In the last case $y=\sqrt{Kx}$.

This is the condition for a maximum of osmotic pressure and not a minimum, for when y is small, λ has its largest value. An increase in y will therefore cause $\frac{de}{dy}\left(\text{and hence } \frac{dP_2}{dy}\right)$ to have at first decreasing positive

values, then to pass through zero, and finally to have decreasing negative values. There will thus be successively a rise and fall of the osmotic pressure with increasing concentration of acid.

II. *Addition of a Monoacid Base to a Non-Diffusible Acid.*—The equations are derived in the same way as those already given. Representing the ratio k_w/k_a by K', we have

$$\frac{1}{\lambda}=\frac{u}{v}=\frac{y}{x}=\frac{v+w-y}{u-x}=\frac{v+w}{u};$$

therefore

$$\frac{1}{\lambda}=\sqrt{1+\frac{w}{v}}.$$

Also

$$\frac{w}{c-w}=\frac{v}{K'} \quad \text{or} \quad w=\frac{cv}{K'+v};$$

therefore

$$\frac{1}{\lambda}=\sqrt{1+\frac{c}{K'+v}}.$$

The last equation shows that as the alkalinity is increased the value of $1/\lambda$ must decrease. Hence λ must increase, approaching 1 as limit.

The equations for osmotic pressure have exactly the same form as those for a non-diffusible base.

III. *Addition of a Monobasic Acid to a Non-Diffusible Ampholyte.*—Here the values of m and n are zero, while $p=1$. Equation (12) takes the form

$$\lambda=\frac{x}{y}=\frac{v}{u}=\frac{y+z-w-v}{x-u}=\frac{y+z-w}{x}.$$

Replacing x by λy

$$\lambda=\sqrt{1+\frac{z-w}{y}} \quad . \quad . \quad . \quad (21)$$

If equations (13) and (14) are solved for z and w, k_w/y substituted for v, and K for k_w/k_b, we have

$$z=\frac{k_b cy}{k_b y+k_w+k_a v}=\frac{cy^2}{y^2+Ky+Kk_a}$$

and

$$w=\frac{k_a cv}{k_a v+k_w+k_b y}=\frac{Kk_a c}{y^2+Ky+Kk_a};$$

hence

$$z-w=\frac{c(y^2-I^2)}{y^2+Ky+I^2} \quad . \quad . \quad (22)$$

where $I^2=Kk_a=\frac{K_a K_w}{k_b}$. From equations (21) and (22)

$$\lambda=\sqrt{1+\frac{c(y^2-I^2)}{y(y^2+Ky+I^2)}} \quad . \quad . \quad (23)$$

Equation (23) indicates that at the isoelectric point, *i.e.* when $y=I$, $\lambda=1$. In the presence of acid ($y>I$), $\lambda>1$. If the acid is in sufficient excess, so that I^2 becomes negligible compared with y^2, then

$$\lambda=\sqrt{1+\frac{c}{K+y}},$$

which is equation (18). (Note that equation (23) also reduces to equation (18) if $k_a=0$.) Hence in excess acid λ decreases with increase in y and again approaches 1 as limit. We thus see that as acid is added in increasing amounts to the pure ampholyte solution, λ must rise from 1 to a maximum and then decrease toward 1. The membrane potentials must rise and fall in a corresponding manner.

The value of y corresponding to the maximum value of λ can be calculated by differentiating equation (23) and putting the derivative equal to zero. The condition proves to be

$$y^4-I^4=4I^2y^2+2I^2Ky \quad . \quad . \quad (24)$$

and this can be solved graphically for y if the values of k_a, k_b, and k_w are known.

The osmotic pressure relation is

$$P_1 = RTe = RT(c + 2y + z - w - 2x).$$

Taking this together with equation (21), we have

$$e - c = y(\lambda - 1)^2,$$

which is equation (20).

Hitchcock gives the complete equation of condition for the maximum of osmotic pressure for an ampholyte with added acid, but remarks that it is too complex to be of any use. The problem is simplified, however, if the maximum occurs at such a high concentration of hydrogen ion that $w = 0$, a condition which makes equation (21) equivalent to equations (16) and (18). If this is the case, then $y = \sqrt{Kx}$, $\log K = 2 \log y - \log x$, or $pK = 2py - px$ (where p is Sørensen's symbol for $-\log$).

IV. *Addition of a Monoacid Base to a Non-Diffusible Ampholyte.*—In this case $q = 1$, while the concentration of the ion A^{p-} is zero. Hence $w + v = y + z + n$ and $u = x + m$. By suitable substitution in equation (12) and combining the result with equation (22) we get the required relation

$$\frac{1}{\lambda} = \sqrt{1 + \frac{cy(I^2 - y^2)}{Kw(y^2 + Ky + I^2)}}.$$

It follows from this equation that with increasing concentration of alkali, λ decreases from 1, passes through a minimum and again approaches 1. The equation of condition for the minimum value of λ is

$$z^4 + 2Ky^3 + 4I^2y^2 = I^4.$$

The osmotic pressure is given by

$$P_1 = RTe = RT(c + 2v + w - z - 2u).$$

From equation (12)

$$v+w-z=\frac{v}{\lambda^2}.$$

Hence

$$e-c=v\left(\frac{1}{\lambda^2}-\frac{2}{\lambda}+1\right)=v\left(\frac{1}{\lambda}-1\right)^2.$$

If the maximum osmotic pressure occurs at such a high alkalinity that z becomes negligible, it can be shown (by reasoning similar to that used in Section I) that the condition for the maximum of osmotic pressure is then

$$\frac{1}{\lambda}=\frac{v}{K'},\quad \lambda=\frac{y}{k_a},\quad \text{or}\quad y=\sqrt{k_a x}.$$

V. *Addition of Acid and Salt to a Non-Diffusible Base.*—Consider first the addition to the non-diffusible base ROH of a monobasic acid (HCl), and salts having the same anion but cations of variable valency q. In this case $p=1$ and $w=0$; hence

$$\lambda=\frac{x}{y}=\frac{v}{u}=\sqrt[q]{\frac{m}{n}}=\frac{y+z+n-v}{x+m-u}=\frac{y+z+n}{x+m}.$$

The following are examples of concrete cases:—

ROH, HCl $\quad \lambda=\sqrt{1+\frac{z}{y}}=\sqrt{\frac{y+z}{y}}$ . . (16)

ROH, HCl, NaCl $\quad \lambda=\sqrt{1+\frac{z}{y+n}}=\sqrt{\frac{y+z+n}{y+n}}$. (25)

ROH, HCl, $CaCl_2$ $\quad \lambda=\sqrt{\frac{y+z+n}{y+\lambda n}}=\sqrt{\frac{y+z+n}{y+\sqrt{mn}}}$. (26)

ROH, HCl, $LaCl_3$ $\quad \lambda=\sqrt{\frac{y+z+n}{y+\lambda^2 n}}=\sqrt{\frac{y+z+n}{y+\sqrt[3]{m^2 n}}}$ (27)

Comparison of the values of λ for systems with identical

values of y and n reveals that the addition of any salt decreases the value of λ.

When sulphuric acid and sulphates are employed instead of hydrochloric acid and chlorides, $p=2$, and equation (12) becomes

$$\lambda=\frac{x}{y}=\frac{v}{u}=\sqrt[q]{\frac{m}{n}}=\sqrt{\frac{y+z+n-v}{x+m-u}}.$$

Neglecting hydroxyl ion concentrations, we obtain the following expressions for the ion ratios:—

ROH, H_2SO_4	$\lambda=\sqrt[3]{1+\frac{z}{y}}=\sqrt[3]{\frac{y+z}{y}}$
ROH, H_2SO_4, Na_2SO_4	$\lambda=\sqrt[3]{1+\frac{z}{y+n}}=\sqrt[3]{\frac{y+z+n}{y+n}}$
ROH, H_2SO_4, $MgSO_4$	$\lambda=\sqrt[3]{\frac{y+z+n}{y+\lambda n}}=\sqrt[3]{\frac{y+z+n}{y+\sqrt{mn}}}$
ROH, H_2SO_4, $La_2(SO_4)_3$	$\lambda=\sqrt[3]{\frac{y+z+n}{y+\lambda^2 n}}=\sqrt[3]{\frac{y+z+n}{y+\sqrt[3]{m^2n}}}.$

It will be observed that the expressions under the radicle signs are similar to those of the preceding group of equations. In both groups these expressions differ with the valence of the salt cation and theoretically there must be some variation in λ due to this cause. Such variation must, however, be slight compared with the difference produced by taking the cube root instead of the square root, which is due to the valence of the added anion.

If p is the valence of the common anion and q that of the salt cation, the above eight equations assume the general form

$$\lambda=\sqrt[p+1]{\frac{y+z+n}{y+\lambda^{q-1}n}}=\sqrt[p+1]{\frac{y+z+n}{y+\sqrt[q]{m^{q-1}n}}}.$$

Condition for Donnan Equilibrium

Before passing on to the experimental side of the subject, attention should be called to the following points. It will be seen from the theory of the Donnan equilibrium that a membrane is not essential. As long as at least one ionic component of a system is kept by some means from diffusing freely to some part of the system the necessary condition has been provided. Instances of Donnan equilibria in the absence of a membrane will be discussed in later sections. It follows that the manner in which a membrane exercises a restraint is immaterial. Thus the non-diffusibility of the particular ion involved may be due to its own large dimensions, or to the formation of ionic aggregates (as in the case of colloidal electrolytes), or to adsorption on the surfaces of colloidal particles, or to some peculiarity in the nature of the ion with respect to the membrane. It is evident that if diffusion through the membrane is very slow the condition for the establishment of a Donnan equilibrium is fulfilled for all practical purposes.

CHAPTER II

SIMPLE CHEMICAL APPLICATIONS

THE investigations on relatively simple systems described in this part of the book are of importance from several points of view. As will be evident, they demonstrate beyond doubt that the distribution of diffusible electrolytes in membrane equilibrium is regulated by the presence of non-diffusible electrolytes in the manner deduced by Donnan. They also illustrate how Donnan equilibria may be employed in the study of various types of disperse systems. Such equilibria, on the one hand, give some idea of the composition, state of aggregation, valency, etc., of the non-diffusible constituents, and, on the other, yield information with regard to the inter-ionic, adsorptive, and other influences to which the simple ions present are subjected. In addition, the investigations provide striking proof of the existence of ionisation in solution, and it appears remarkable that equilibria at membranes were not more fully investigated at an early stage in the development of the theory of Arrhenius.

FERROCYANIDES

The first investigation dealing quantitatively with the distribution relations derived by Donnan was carried out by Donnan and Allmand (15), who made use of the fact that a membrane of cupric ferrocyanide is permeable to chlorides but not to ferrocyanides. A solution of $K_4Fe(CN)_6$ was placed on one side (1) of the membrane and KCl on the other (2), and the equilibrium distribu-

tion of the chloride determined by analytical means. In agreement with theory (Case i) the concentration of chloride in (2) was always greater than in (1), and the "expelling" action exerted by the ferrocyanide on the chloride, measured by $[Cl]_2/[Cl]_1$, increased with increase in the ratio of ferrocyanide to chloride. In the extreme case examined the concentration of potassium chloride in compartment (2) was, at equilibrium, three times that in compartment (1). Kameyama (11) has also made a study of the same system, and his results in the main confirm the data of Donnan and Allmand.

The equation of equilibrium may be written

$$f_1 m_1 = f_2 m_2 \qquad \text{(equation 11)}.$$

Now m_1 and m_2 are known from the chemical analyses of the equilibrium solutions, and the value of f_2 (the mean activity coefficient in the pure KCl solution) can be obtained by interpolation from existent data. Hence, assuming the equation to hold, we may calculate the value of f_1. According to G. N. Lewis (16), in sufficiently dilute solution the activity coefficient of a given strong electrolyte is the same in all solutions of the same "ionic strength" μ, where μ is obtained by multiplying the molarity of each ion by the square of its valency and taking half the sum of the resultant products. The value of μ_1 in the mixed solution in (1) is given by

$$\mu_1 = \tfrac{1}{2}(m_K + m_{Cl} + 4^2 \cdot m_{Fe(CN)_6}) = m_{Cl} + 2{\cdot}5 n_{Fe(CN)_6},$$

since

$$m_K = m_{Cl} + 4m_{Fe(CN)_6} = m_{Cl} + n_{Fe(CN)_6}$$

where n denotes the normality of the potassium ferrocyanide. Hence if the ionic strength principle holds in

the present case, we should expect that f_1, obtained from

$$f_1 = \frac{m_2}{m_1} f_2,$$

will be equal to f_0, the activity coefficient of potassium chloride in a pure aqueous solution of this salt of ionic strength $= \mu_1$.

Kameyama found that the results of his own experiments agreed well with the ionic strength principle. In the great majority of cases the divergence of f_1/f_0 from unity was not more than about 2 per cent., and in no case greater than about 5 per cent. Kameyama also calculated the values of f_1/f_0 corresponding to the data of Donnan and Allmand. The agreement was again good, except for a few experiments in which the experimental error was probably large.

Mention should also be made of Hückel's use of the data of Donnan and Allmand. Hückel (10) has shown that these, apart from the doubtful figures (at low concentrations of chloride), lead to values of f_1 which fit the expression

$$\log f = \frac{-0{\cdot}351\sqrt{\Gamma}}{1 + 3{\cdot}86 \times 0{\cdot}231 \,.\, 10^8\sqrt{\Gamma}}$$

where Γ is equal to twice the ionic strength. The formula was deduced by the application of Debye's theory (17), and is almost identical with the formula which expresses the behaviour of solutions containing potassium chloride alone.

An example of Case (ii) (see p. 5) has been investigated by Donnan and Garner (7). Solutions of potassium and sodium ferrocyanides, of unequal concentrations, were placed on opposite sides of a copper ferrocyanide membrane and the equilibrium distribution

of the sodium and potassium determined. It was found that the ratio of total sodium to total potassium was, within the small experimental error, the same on both sides of the membrane. The exact form of the distribution equation is

$$(a_{Na})_1(a_K)_2=(a_{Na})_2(a_K)_1,$$

or

$$(f_{Na}m_{Na})_1(f_Km_K)_2=(f_{Na}m_{Na})_2(f_Km_K)_1.$$

It would therefore appear that the activity coefficients of the sodium and potassium ions are very similar, which is in line with other evidence.

The experiments of Donnan and Garner (7), in which potassium ferrocyanide was replaced by calcium ferrocyanide, are of particular interest since a divalent diffusible ion is involved (see p. 7, Case iii). As the following table demonstrates, the relation between the total concentrations of Ca and Na is closely expressed by the formula

$$\frac{[Ca]_1}{[Ca]_2}=\frac{[Na]_1^2}{[Na]_2^2}.$$

TABLE IV.—DISTRIBUTION RATIOS

$\frac{[Ca]_1}{[Ca]_2}$	$\frac{[Na]_1^2}{[Na]_2^2}$	$\frac{[Na]_1}{[Na]_2}$
1·265	1·248	1·117
1·762	1·757	1·326
1·368	1·332	1·154
1·802	1·775	1·335
1·547	1·495	1·223

The differences between columns one and two are of the same order as the errors of analysis. Since the

distribution equation is

$$\frac{(a_{Ca})_1}{(a_{Ca})_2}=\frac{(a_{Na})_1^2}{(a_{Na})_2^2},$$

it follows that

$$\frac{(f_{Ca})_1}{(f_{Ca})_2}=\frac{(f_{Na})_1^2}{(f_{Na})_2^2}.$$

Some work has been done on membrane potentials in systems of ferrocyanides. Kameyama (18) measured the electromotive force of cells of the type

$$\begin{array}{c}\text{Potassium amalgam, } K_4Fe(CN)_6 \\ (m_1)\end{array} \Big| \begin{array}{c}K_4Fe(CN)_6\text{, Potassium amalgam} \\ (m_2)\end{array}$$

where | represents a copper ferrocyanide membrane and m_1 and m_2 the molarities of the solutions separated by it. As the electrodes were made from the same sample of amalgam, the system in the absence of membrane constitutes a concentration cell, the electromotive force of which is given by

$$E_c=\frac{RT}{\mathbf{F}}\ln\frac{(a_K)_1}{(a_K)_2}=\frac{RT}{\mathbf{F}}\ln\frac{(m_K f_K)_1}{(m_K f_K)_2}.$$

Now the membrane potential E_m is equal to E_c in magnitude but is of opposite sign (see p. 16). Consequently E, the total electromotive force of the chain, should be equal to zero, provided that conditions at the membrane are as simple as postulated. Actually the value of E was found to be less than a millivolt except when a solution more dilute than 0·005 molar was involved, in which case it appeared probable that disturbing effects were produced by the interaction of the amalgam with the water.

Donnan and Green (19) have studied the combinations

$$\begin{array}{cccccc}\text{(A)} & \text{Cal., } K_4Fe(CN)_6 & | & K_4Fe(CN)_6\text{, Cal.} \\ & e_1 \quad (1) & E_m & (2) \quad e_2\end{array}$$

(B) Cal., $K_4Fe(CN)_6$, $K_4Fe(CN)_6$, Cal.
e_1 (1) E_d (2) e_2

the abbreviation Cal. denoting calomel electrodes. The electromotive force of (A) is made up of the three potential differences e_1, e_2, and E_m. In (B) the membrane is absent and E_m is replaced by the ordinary diffusion potential E_d between the solutions (1) and (2). If e denotes the difference between the electromotive forces of (A) and (B), then $e = E_m - E_d$ and therefore $E_m = e + E_d$. In order to find E_m it is therefore necessary to calculate E_d. For this purpose Donnan and Green used an equation of the usual type, involving transport numbers and degrees of ionisation, values for which were obtained from conductivity data. The values of E_m arrived at in this fashion are to a considerable extent uncertain, but they may be compared with those calculated by a method suggested by Kameyama (18). Kameyama assumes that f_K in a solution of $K_4Fe(CN)_6$ is equal to $f_{KCl} (= \sqrt{f_K f_{Cl}})$ in a solution of KCl of the same ionic strength. Hence values for f_K in the formula

$$E_m = \frac{RT}{\mathbf{F}} \ln \frac{(f_K m_K)_1}{(f_K m_K)_2}$$

are obtained by interpolation on the $\mu - f_{KCl}$ curve for pure solutions of KCl. At the lower concentrations examined by Donnan and Green the two sets of values for E_m are fairly close, but they diverge considerably at the higher concentrations. In other words, as might be expected owing to the uncertainty in E_d, the deviation increases with the proportion that E_d plays in the determination of E_m. The agreement is, however, sufficient to prove that the values for E_m obtained by Donnan and Green are of the right order of magnitude.

Donnan and Allmand (15) investigated the potentials of cells constituted as follows:—

$$\underset{(1)}{\text{Ag, AgI} + \text{K}_4\text{Fe(CN)}_6 + \text{KI}} \;\Big|\; \underset{(2)}{\text{KI} + \text{AgI, Ag}}$$

where $K_4Fe(CN)_6$ and KI are present in concentrations identical with those of $K_4Fe(CN)_6$ and KCl in the equilibrium systems already discussed (see p. 39) and where the electrodes are reversible to the iodide ion. It was found impossible to obtain satisfactory results in the $K_4Fe(CN)_6 - KCl$ mixtures by using electrodes reversible to the chloride ion. The iodide cells gave definite electromotive forces (1·5 to 9·5 millivolts), which increased as the ratio of ferrocyanide to iodide was made greater. These results suggested that the potassium iodide was not in distribution equilibrium in the cells. On the other hand, cells with potassium amalgam electrodes in place of the silver-silver iodide electrodes showed anomalous behaviour since the observed electromotive force was in no case greater than 1·5 millivolts; and, allowing for the decomposition of the amalgam by the water, the true potential difference could be taken as equal to zero. It is evident that the question requires further investigation.

Colloidal Dyes

As has already been mentioned, Donnan and Harris (2) investigated the distribution of sodium chloride across a parchment membrane with Congo-red on one side. These workers showed quite definitely the existence of unequal distribution at equilibrium, but did not attempt to apply the distribution equations. This, however, has been done by Azuma and Kameyama (20), who found, in agreement with the observations of Donnan and Harris,

that the sodium chloride was always more concentrated on the side free from Congo-red. Moreover, as predicted by theory (see p. 5), the more concentrated the Congo-red the more marked was the inequality of the distribution. Representing the final state by

$Na_2A + NaCl$	NaCl
$c_1 \quad c_3$	c_2
(1)	(2)

(c_1, c_2, c_3 = molar concentrations),

the equilibrium may be written (with respect to NaCl)

$$a_1 = a_2 \text{ (mean activities).}$$

That is to say,

$$f_1 m_1 = f_2 m_2 \text{ (see p. 25)}$$

or

$$f_1 m_1 / f_2 m_2 = 1.$$

In order to apply their analytical data to the testing of this relation, Azuma and Kameyama assumed that at the concentrations concerned Congo-red behaves as a strong uni-univalent electrolyte, *i.e.* that it ionises according to the equation

$$Na_2A = Na^+ + (NaA)^-.$$

Hence m_{Na} was put equal to $c_1 + c_3$. For the evaluation of f_1 it was further assumed that the ionic strength principle held for the mixed solution in (1). Hence f_1 was taken as having the same value as the activity coefficient in a pure solution of sodium chloride of ionic strength (μ_1) equal to

$$1/2(m_{Na} + m_{Cl} + m_{NaA})_1 = c_1 + c_3.$$

In order to obtain actual values for f_1 and f_2, the $f - \mu^{\frac{1}{2}}$ curve for sodium chloride was drawn from data given by Lewis and Randall (16), and values of f interpolated at

the experimental values of the ionic strength. Since $m_{Na}=m_{Cl}=c_2$ and $m_{NaA}=c_3$, the values of m_{Na} and m_{Cl} in (2) and of m_{NaA} could be obtained directly from the analyses of the equilibrium solutions. Azuma and Kameyama employed a membrane of collodion. Since this adsorbs Congo-red to a considerable extent, the concentration of the dye was determined after equilibrium was reached. Table V shows the results obtained.

TABLE V.—DISTRIBUTION OF SODIUM CHLORIDE IN PRESENCE OF CONGO-RED *

c_1	c_3	c_2	c_3/c_2	$100c_1/c_3$	a_1/a_2	a'_1/a'_2
2·0	50·2	51·0	0·984	4·0	1·002	1·016
3·1	28·4	29·6	0·959	10·9	1·007	1·043
4·3	32·4	34·0	0·953	13·3	1·010	1·053
3·9	28·0	29·9	0·937	13·9	0·995	1·040
5·5	19·3	21·3	0·906	28·5	1·019	1·099
4·8	14·7	16·3	0·902	32·7	1·028	1·121
5·3	15·1	17·0	0·888	35·1	1·023	1·120
5·9	12·5	14·9	0·839	47·2	1·008	1·122
6·0	10·5	12·2	0·861	57·2	1·064	1·204
6·0	6·4	8·0	0·800	93·8	1·093	1·288

* Concentrations expressed as millimols per litre.

The figures in the last column are the ratios of the mean activities calculated on the assumption that Congo-red ionises completely according to the equation

$$Na_2A=2Na^+ + A^-.$$

It is obvious that a'_1/a'_2 deviates more widely from unity than a_1/a_2. On the other hand, while the deviation in the latter is generally less than 3 per cent., there seems to be a distinct tendency for this deviation to increase as the ratio $100c_1/c_3$ becomes larger.

Azuma and Kameyama also attempted to determine the membrane potentials of the systems described above, by means of cells similar to those used by Donnan and Green (19), except that bridges of gelatine jelly containing NaCl were employed to connect the solutions with the calomel electrodes. The observed values of E_m were very much smaller than those calculated from the distribution data, and Azuma and Kameyama concluded that this was probably due to the setting up of a membrane potential at the junction of the gel and solution (2) owing to the extreme slowness with which the Congo-red diffused into the gel.

Donnan and Harris (4) observed that the apparent osmotic pressure of an aqueous solution of Congo-red (contained in a parchment paper osmometer immersed in water) rose relatively quickly to a maximum and then slowly fell. If the outside water was now renewed, the pressure again rose and fell in similar fashion, and this behaviour was repeated with each change of water. It was also found that as the experiment progressed the amount of sodium in the osmometer gradually diminished and the contents of the osmometer became brown to violet in colour and appeared to form a colloidal suspension. Addition of NaOH restored the original bright red colour of the solution. Evidently the Congo-red was undergoing membrane hydrolysis, NaOH diffusing out and leaving an insoluble acid or acid salt. The initial increase in pressure following a renewal of the outside water was thus due to the removal of the counter pressure caused by the hydrolysis alkali, and the subsequent decrease to the further formation of alkali. As was therefore to be expected, the suppression of hydrolysis by the addition of NaOH to the Congo-red led to steady values for the pressure. In agreement with

theory (see p. 22) it was found that a very small quantity of alkali was sufficient for the purpose.

The hydrolysis will be promoted by the presence of carbonic acid in the outer liquid (through neutralisation of the alkali), which explains the statement of Bayliss (21) that CO_2 lowers the osmotic pressure of Congo-red.

As previously noted by Bayliss (21) and Biltz and Vegesack (22), Donnan and Harris found that an apparent lowering of the osmotic pressure of Congo-red was produced by the addition of NaCl or NaOH. Their experiments led them to the conclusion that this effect could be satisfactorily explained on the basis of the unequal distribution of the diffusible electrolyte across the osmometer membrane (see p. 19). There was therefore no need to postulate a decrease in the degree of dispersion of the dyestuff due to some action of the added electrolyte.

Mention should be made of some experiments carried out by Biltz (23), which demonstrate the production of unequal distribution of diffusible electrolytes by the action of the dyestuffs Kongoreinblau and Brillantkongo.

The most recent investigation of membrane equilibria in systems of colloidal dyes is that of Robinson and Mills (24) on Benzopurpurin-4B and its isomer prepared from *m*-tolidine. Since the osmotic pressures (measured in the presence of sufficient alkali to check membrane hydrolysis) of the two dyes were found to be almost the same, it might be concluded that in solution these substances form colloidal micelles of the same size. Robinson and Mills point out that this is not necessarily the case. Suppose, for example, two dyes (*a*) and (*b*) dissociate as follows:—

(*a*) $200Na^+ + 10(A)_{10}^{20-}$

(*b*) $200Na^+ + 5(A)_{20}^{40-}$.

Their osmotic pressure would be as 210 to 205, a difference of only 2·5 per cent., though the micelle of (*b*) is twice the size of the micelle of (*a*).

It is obviously necessary to determine the osmotic pressure due to the non-diffusible ion alone. This may be effected by measuring the osmotic pressure in the presence of a certain concentration of NaCl. Suppose the dye dissociates in the following manner:—

$$n\mathrm{NaA} \rightleftharpoons n\mathrm{Na} + \mathrm{A}^{n-},$$

then the initial and final conditions in the simplest case (equal volumes of liquid) are

Initial				Final			
nc_1	Na^+	Na^+	c_2	nc_1+x	Na^+	Na^+	c_2-x
c_1	A^{n-}	Cl^-	c_2	c_1	A^{n-}	Cl^-	c_2-x
				x	Cl^-		
(1)		(2)		(1)		(2)	

If P_0=true osmotic pressure of NaA and P=opposing pressure due to the unequal distribution of the sodium chloride, the observed pressure is given by

$$P_1 = P_0 - P = (n+1)c_1RT - (2c_2 - 4x)RT \; . \qquad (28)$$

This is the general form of equation (8) (p. 20). From equation (28) and the Donnan relation

$$(nc_1+x)x = (c_2-x)^2,$$

we have

$$\frac{P_1}{P_0} = \frac{c_1 + \frac{2}{n(n+1)}c_2}{c_1 + \frac{2}{n}c_2}.$$

This reduces to equation (9) when $n=1$. As c_2 becomes very large compared with c_1, P_0/P_1 approaches the limiting value $n+1$, or, in other words, we are measuring the osmotic pressure due to the non-diffusible ions.

It was found by Robinson and Mills that, in the case of the "meta" dye, the limiting value of P_0/P_1 was about 21 (in 0·25 M NaCl). This would indicate that $n=20$, *i.e.* that the dye micelle contains 10 disulphonate anions. Robinson and Mills use this value of n to calculate the ratio P_0/P_1 in the presence of 0·0143 M sodium chloride, when nc_1 could be taken as approximately equal to c_2. Since in this case the ratio of the volume of liquid in (2) to the volume in (1) was 50, we have (in arbitrary units)

Initial				Final			
20	Na^+	Na^+	20	$20+50x$	Na^+	Na^+	$20-x$
1	A^{n-}	Cl^-	20	1	A^{n-}	Cl^-	$20-x$
				$50x$	Cl^-		
(1)		(2)		(1)		(2)	

Then

$$P_0=21RT,$$
$$P=[2(20-x)-100x]RT,$$
$$P_1=P_0-P=(102x-19)RT.$$

From Donnan's theory, at equilibrium

$$(20+50x)50x=(20-x)^2$$
$$x=0{\cdot}243.$$

Hence

$$P_1=(24{\cdot}78-19)RT=5{\cdot}78RT,$$

and

$$P_0/P_1=21/5{\cdot}78=3{\cdot}63.$$

Since 4·4 was found, it would seem that n must be greater than 20. Robinson and Mills remark that it is very improbable that 0·0143 M sodium chloride is able to bring about this state of aggregation, and that, while present data cannot give the exact value of n, the evidence points to a micelle of 10 (or more) anions.

Robinson and Mills attempt to calculate the value of the activity of the sodium ion of the "meta" dye from the equilibrium distribution of the sodium chloride. They assume complete ionisation, and that the activity coefficient of the sodium ion due to the NaCl in $(1)=(f_{Cl})_1=(f_{Cl})_2$. It was found that the calculated value increased as the concentration of NaCl was increased, which appeared to show that the above simplification introduced large errors.

Inorganic Colloids

The Donnan equilibrium provides a method for the measurement of the adsorption of ions by the particles in inorganic colloidal systems. Determinations of this kind have been carried out by Bjerrum (25), Rinde (26), and Ganguly and Krishnamurti (27). In Rinde's experiments a sulphur sol (formed by the interaction of $Na_2S_2O_3$ and H_2SO_4 and dialysed) was placed in a collodion bag immersed in dilute HCl solution. At equilibrium the activity of the hydrogen ions (obtained by means of the quinhydrone electrode) was found to be greater inside than outside the bag. Moreover, the variation of the inequality of the distribution with change in the concentration of the acid showed that as the latter increased the negative charge on the sulphur particles also increased. This effect must be due to the adsorption of chlorine ions by the particles, the chlorine ions thereby becoming non-diffusible. The extent of the adsorption was evaluated as follows:—

$$(a_H)_1(a_{Cl})_1=(a_H)_2(a_{Cl})_2.$$

Also

$$(a_{Cl})_1=(f_{Cl})_1[Cl^-]_1=(f_{Cl})_1([H^+]_1-z),$$

where z=concentration of adsorbed chlorine ions and

$(f_{Cl})_1$ = activity coefficient of the chlorine ion inside the membrane. Hence

$$z=[H^+]_1-\frac{(a_H)_2(a_{Cl})_2}{(f_{Cl})_1(a_H)_1},$$

$$=\frac{(a_H)_1}{(f_H)_1}-\frac{(a_H)_2(a_{Cl})_2}{(f_{Cl})_1(a_H)_1}.$$

Since it is probable that the activity coefficients of the hydrogen and chlorine ions in pure aqueous solutions of hydrochloric acid are not very different, we may put $(a_H)_2=(a_{Cl})_2$. It may further be assumed that if the concentration of non-diffusible ions is low, $(f_H)_1$ and $(f_{Cl})_1$ can be replaced by a mean value f, which is equal to the activity coefficient of the acid outside the membrane. If we put $(a_H)_2=(a_{Cl})_2=a_2$ and $(a_H)_1=a_1$, the final equation is

$$z=\frac{a_2}{f}\left(\frac{a_2}{a_1}-\frac{a_1}{a_2}\right).$$

The membrane potential is, of course, given by

$$E_m=\frac{RT}{\mathbf{F}}\ln\frac{a_1}{a_2}.$$

Rinde records that, for no apparent reason, he often failed to obtain a measurement of the membrane potential. When, however, the value of E_m was constant, it agreed with that predicted by the formula, which showed that the systems were really in equilibrium. In order to make a satisfactory measurement it was found necessary to ensure that the pressure of the solution inside the membrane against that outside was the same as the osmotic pressure at equilibrium.

Assuming complete ionisation of the hydrochloric acid, it might be expected that the osmotic pressure

difference between the two sides of the membrane would be calculable, at least approximately, from the relation

$$P_1 = RT(c + [H^+]_1 + [Cl^-]_1 - 2[H^+]_2),$$

where c = concentration of colloidal particles ($c = 1$ when the number of particles per litre = Avogadro's constant) and will have a relatively small value. The osmotic pressure actually observed by Rinde was, however, much less than the value of $RT([H^+]_1 + [Cl^-]_1 - 2[H^+]_2)$. Rinde suggests that a possible explanation may be found in a theory advanced by H. Hammarsten (28) to account for the similar state of affairs encountered in a research on the osmotic pressure of certain electrolytes with large molecules. The theory is that a colloidal ion and an ordinary ion behave osmotically as a single individual unless the distance between them exceeds a certain critical value.

If the colloidal particles adsorb the hydrogen ion from HCl solution, the formula to be employed is

$$z = \frac{a_2}{f}\left(\frac{a_2}{a_1} - \frac{a_1}{a_2}\right).$$

Ganguly and Krishnamurti (*loc. cit.*) have applied this relation to the case of colloidal silicic acid. Bjerrum's paper (*loc. cit.*) deals with the adsorption of acid by colloidal chromic hydroxide. It contains a number of errors; these have been discussed by Rinde (*loc. cit.*).

Ghosh (29) makes use of the Donnan equilibrium to investigate the action of sodium hydroxide on α-stannic acid sol.* The mixture of colloid and alkali was placed on one side (1) of a parchment or collodion membrane, water being on the other, and the membrane potential

* See Ganguly (30) for the similar case of the distribution of sodium silicate solutions across a membrane.

measured. Analyses of the equilibrium liquids showed that both sodium and tin were present in the water chamber. If it be assumed that the diffusible tin is ionised $NaHSnO_3$, the equilibrium equations are

$$(a_{Na})_1/(a_{Na})_2=(a_{HSnO_3})_2/(a_{HSnO_3})_1=(a_{OH})_2/(a_{OH})_1=\lambda$$

and

$$E_m=\frac{RT}{\mathbf{F}}\ln\lambda.$$

The initial value of the ratio SnO_2/Na_2O was varied over the range 0·5 to 17·2. Even at the lowest ratio the amount of stannic oxide in the sol chamber was, at equilibrium, about twelve times that in the water chamber, indicating that most of the SnO_2 was in the colloidal condition. At the higher ratios there was a considerable excess of total alkali in the sol chamber, which means that some sodium combined with the sol particles. It was found, however, that the ratio of the sodium ion activities in (1) and (2), derived from the membrane potential, was much less than the ratio of total Na_2O as determined by titration. This showed that the dissociation of the colloidal complex was small. Assuming that the $NaHSnO_3$ is completely ionised and that the osmotic pressure of the colloid is negligible, the observed pressure will be (according to the simple theory) approximately equal to

$$RT([Na^+]_1+[HSnO_3^-]_1+[OH^-]_1 \\ -[Na^+]_2+[HSnO_3^-]_2+[OH^-]_2).$$

Ghosh found that this expression could be applied when the initial ratio of SnO_2 to Na_2O was 4·5 or more, but that at lower ratios the calculated pressures were much too small.

It may be pointed out that Ghosh is in error with regard to the theory of the membrane potential. On

p. 2295 of his first paper he states that the assumption that all the diffusible ions present in his systems are univalent is justified by the equality which exists between the membrane potential as directly observed and as calculated from the expression,

$$E=\frac{RT}{\mathbf{F}}\ln\frac{(a_{OH})_2}{(a_{OH})_1},$$

using values for the hydroxyl-ion activities deduced from measurements with the hydrogen electrode. In view of the discussion on p. 18 of this book it is obvious that this argument is not valid.

Proteins

Various investigations have shown (see Lewis (31), Jordan-Lloyd (32), and Hitchcock (33), (40)) that when hydrochloric acid is added to a solution of a protein (in the isoelectric condition), the hydrogen ions are taken up by the protein to a marked extent, but that the chlorine ions remain practically uninfluenced in any way by the protein, provided the concentration of free hydrogen ions does not exceed about 0·01 M. Hence the acidified solution of protein consists essentially of neutral and positively charged colloidal units in equilibrium with free hydrogen and chlorine ions. It is therefore to be expected that if such a solution is separated from water by a membrane impermeable to the colloid only, the acid will be distributed unequally across the membrane when equilibrium is attained. Representing the equilibrium state by

$$\begin{array}{cccc|cc} B & B^{n+} & H^+ & Cl^- & H^+ & Cl^- \\ & & (1) & & & (2) \end{array}$$

where B=protein, we should have

$$[H^+]_2>[H^+]_1 \quad \text{and} \quad [Cl^-]_1>[Cl^-]_2.$$

J. Loeb and his co-workers (34), (35), (37) have repeatedly demonstrated that such an equilibrium state is actually established.

The distribution equation may be put in the form,

$$\log \frac{(a_H)_2}{(a_H)_1} = \log \frac{(a_{Cl})_1}{(a_{Cl})_2}.$$

In some experiments by Loeb (34), (37) on 1 per cent. solutions of gelatin (contained in collodion sacs), the logarithms of the activity ratios were obtained by means of the cells

Pt, H_2, solution (1) or (2), KCl (satd.), Cal. (satd.)
Hg, solution (1) or (2) satd. with Hg_2Cl_2, KCl (satd.), Cal. (satd.).

Table VI contains the results and shows clearly the existence of an equilibrium state of unequal distribution.

TABLE VI.—INFLUENCE OF GELATIN ON THE DISTRIBUTION OF HYDROCHLORIC ACID

pH of gelatin solution at equilibrium	$\log \frac{(a_H)_2}{(a_H)_1}$	$\log \frac{(a_{Cl})_1}{(a_{Cl})_2}$
4·31	0·56	0·48
3·69	0·58	0·51
3·30	0·50	0·59
3·10	0·49	0·44
2·92	0·44	0·44
2·78	0·44	0·38
2·46	0·33	0·35
2·26	0·23	0·22
2·01	0·15	0·15
1·76	0·10	0·11

Loeb studied the variation of $\log \frac{(a_H)_2}{(a_H)_1}$ with increasing

acidity. As Table VII illustrates, this quantity rises to a maximum and then decreases.

TABLE VII.—VARIATION IN pH (1) – pH (2) WITH CONCENTRATION OF ACID

C.c. 0·1 M HCl added to 100 c.c. 1 per cent. isoelectric gelatin	pH of (1)	$\log \frac{(a_H)_2}{(a_H)_1}$ (pH of (1) – pH of (2))	$y \cdot 10^5$	$z \cdot 10^5$	z/y
1	4·56	0·42	2·7	16·5	6·1
2	4·31	0·53	4·9	51·4	10·5
4	4·03	0·59	9·3	132·5	14·3
6	3·85	0·59	14·1	200·0	14·2
8	3·33	0·46	46·8	343·0	7·3
10	3·25	0·44	56·2	372·0	6·6
12·5	2·85	0·32	141·0	477·0	3·4
15	2·52	0·24	302·0	608·0	2·0
20	2·13	0·13	741·0	609·0	0·82
30	1·99	0·10			
40	1·79	0·07			
50	1·57	0·04			

This variation of pH (1) – pH (2) may be interpreted as follows (see Donnan (8), Loeb (34) and (35)). In terms of molar concentrations we have at equilibrium,

(1)		(2)	
z	$(B)H^+$		
y	H^+	H^+	x
$y+z$	Cl^-	Cl^-	x

where z = concentration of bound hydrogen ions. Since the conditions of the experiments were such that the activity coefficients of the diffusible ions may be re-

garded as the same in (1) and (2), we may apply the relations:

$$x^2=y(y+z),$$

$$x=\sqrt{y(y+z)},$$

$$\frac{[H^+]_2}{[H^+]_1}=\frac{x}{y}=\frac{\sqrt{y(y+z)}}{y}=\sqrt{\frac{y+z}{y}}=\sqrt{1+\frac{z}{y}},$$

$$z=\frac{(x^2-y^2)}{y}=\frac{(x+y)(x-y)}{y}.$$

When $x=0$, z also $=0$, and $1+\frac{z}{y}=1$. On the addition of acid both y and z increase, but z increases more rapidly than y when the concentration of acid is low. Hence the value of $1+\frac{z}{y}$ at first increases with increase in acidity. At higher concentrations of acid, however, while y continues to increase, z reaches a limiting value since the protein can take up only a certain amount of hydrogen ion. Thus the value of $1+\frac{z}{y}$ attains a maximum and then sinks asymptotically to unity (Table VII). Correspondingly the difference in pH between (2) and (1) will increase from zero, pass through a maximum and then tend toward zero again.

The membrane potential is given by

$$E_m=\frac{RT}{\mathbf{F}}\ln\frac{x}{y}=2{\cdot}303\frac{RT}{\mathbf{F}}[\text{pH}\,(2)-\text{pH}\,(1)],$$

and therefore varies as above. Loeb (37) succeeded in measuring the membrane potentials by means of the cell Cal. (satd.), KCl (satd.), (1) | (2), KCl (satd.), Cal. (satd.) where (1) and (2) were separated by a collodion mem-

brane. In Table VIII will be found the potentials observed directly by Loeb, together with those "calculated" from the pH differences.

TABLE VIII.—COMPARISON OF OBSERVED AND "CALCULATED" POTENTIALS

pH of (1)	E_m Observed (millivolts)	E_m "Calculated" (millivolts)
4·56	24·0	24·7
4·31	32·0	31·0
4·03	33·0	34·5
3·85	32·5	34·5
3·33	26·0	27·0
3·25	24·5	25·8
2·85	16·5	18·8
2·52	11·2	14·0
2·13	6·4	7·6
1·99	4·8	5·9
1·79	3·7	1·4
1·57	2·1	2·3

The agreement between the two sets of values simply indicates that the systems examined were really in the equilibrium state and that the experiments were reasonably accurate. It is perhaps necessary to emphasise this, since in many of Loeb's publications the view appears to be taken that such concordance constitutes *in itself* a proof that the equilibrium is of the Donnan type. Hill (38) has pointed out that provided the state of unequal distribution is a true equilibrium state, it is a thermodynamic necessity that the membrane potential should be equal to $RT/\mathbf{F}$ times $\ln \frac{(a_i)_2}{(a_i)_1}$, for any species of

diffusible ion, quite irrespective of the cause of the inequality in distribution. Hitchcock (39) has also dealt with this question.

On the assumption that the protein is an ampholyte, the variation of the ionic distribution ratio with change in acidity is strictly represented by equation (23) (p. 34). Since, however, the concentrations of protein cations and anions must necessarily have the same small value at the isoelectric point, the problem is essentially that of the neutralisation of a non-diffusible base which ionises to a negligible extent, and the simplified treatment given above becomes applicable (Hitchcock (14) and Donnan (8)).

Hitchcock (40) has shown that the combination curve of gelatin with hydrochloric acid is approximately equivalent to the ionisation curve of a monoacid base having $K=k_w/k_b=2{\cdot}4\times10^{-4}$. This result may be used to make a rough test of equation (24) (see p. 34). I has the value of y at the isoelectric point, *i.e.* $y=2\times10^{-15}$ (pH=4·7). Employing these values of K and I to solve equation (24), we obtain $6{\cdot}7\times10^{-5}$ as the calculated value of y at the point of maximum membrane potential. This is equivalent to a pH of 4·17, which approximates to Loeb's observed value of 4·0.

Loeb and Kunitz (41) have studied the influence of the basicity of the acid on the value of the membrane potential. As will be seen from Fig. 1, all the potentials obtained with monobasic acids lie approximately on one curve, while those with strong dibasic acids lie on a second which has a considerably lower maximum.

As Loeb (34), (35) has pointed out, the Donnan theory affords an explanation of the difference in effect of the two types of acid. The equilibrium equation for the dibasic acid is

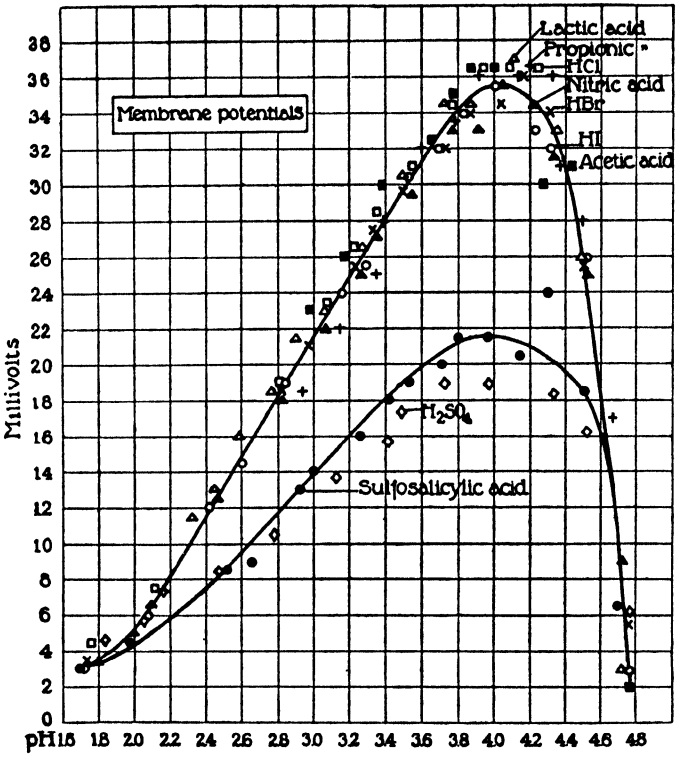


FIG. 1.—Influence of acids on the membrane potentials of gelatin solutions (From Loeb and Kunitz, *Journal of General Physiology*, 1922–1923)

$$x^3 = y(y+z).$$

Hence $x = \sqrt[3]{y(y+z)}$, and $\frac{x}{y} = \sqrt[3]{1+\frac{z}{y}}$.

In this case the membrane potential is therefore given by

$$E_m = \frac{RT}{\mathbf{F}} \ln \sqrt[3]{1+\frac{z}{y}} = \frac{RT}{3\mathbf{F}} \ln \left(1+\frac{z}{y}\right).$$

For a monobasic acid we have

$$E_m = \frac{RT}{2\mathbf{F}} \ln \left(1+\frac{z}{y}\right).$$

Hence at the same pH of the gelatin solution the ratio of the potential difference for the dibasic acid to that for a monobasic acid should be as 2 to 3, or 0·66. A comparison of the potentials for sulphosalicylic acid with those for HCl and the other monobasic acids in Fig. 1 shows that this holds within the limits of experimental accuracy (Table IX).

TABLE IX.—INFLUENCE OF VALENCY ON MEMBRANE POTENTIALS

pH of gelatin solution	Potentials		Ratio
	Dibasic acid (millivolts)	Monobasic acid (millivolts)	
2·4	7·6	11·4	0·67
2·6	9·6	14·8	0·65
2·8	11·6	18·0	0·64
3·0	13·6	21·6	0·65
3·2	15·8	24·8	0·64
3·4	18·0	28·0	0·62
3·6	19·8	31·0	0·64
3·8	21·2	34·2	0·62
4·0	21·6	35·5	0·61
4·2	20·8	34·8	0·60
4·4	19·2	31·0	0·62

The interpretation of the influence of weak dibasic and tribasic acids follows naturally from the foregoing considerations. For example, H_3PO_4 dissociates as a monobasic acid below pH 4·7, and Loeb (37) has shown that over this range of pH the influence of H_3PO_4 on the membrane potential is identical with that of HCl, when comparison is made at the same pH for the protein solution.

Loeb (34), (37) found that the addition of neutral salts

depresses the membrane potential at a membrane separating a solution of gelatin containing HCl from a solution containing the acid alone. Table X illustrates the effects observed.* $NaNO_3$ and the chlorides of Na,

TABLE X.—INFLUENCE OF SALTS ON MEMBRANE POTENTIALS

Equivalent concentration of salt	Observed membrane potential (millivolts)		
	$NaNO_3$	$CaCl_2$	Na_2SO_4
0	31·0	28·6	26·5
1/2048	24·0	23·0	17·0
1/1024	22·0	19·2	12·5
1/512	16·0	14·5	8·4
1/256	12·0	9·1	4·7
1/128	7·0	5·7	3·4
1/64	4·1	3·1	1·5
1/32	0·0	1·8	0·0

Ca, and La produce approximately the same decrease, while Na_2SO_4 is considerably more effective. In every case comparison must of course be made with the system initially the same. The decrease in the potential implies that the presence of the salt diminishes the inequality in the distribution of the diffusible ions. Such an effect is predicted by the theory. We have here in principle an example of the case for which equation (6) holds. This equation shows that as the concentration of the salt is increased, the ionic ratio λ tends toward unity and hence the membrane potential must gradually approach zero.

* According to Loeb (34) the range of salt concentration in Table X is such that no direct influence of the salt on the pH of the gelatin need be considered.

The effect of neutral salts may also be deduced from Hitchcock's equations (see p. 36). Loeb found that the values of E_m were, within the experimental limits, the same for NaCl, $CaCl_2$, and $LaCl_3$ at the same equivalent concentration and pH. Equations (25), (26), and (27) can be identical only when $\lambda=1$ (or $m=n$). There is thus a possibility that some differences due to the valence of the salt cation might become evident as the result of more exact experiment. Hitchcock's equations show that the marked influence of the valence of the salt anion (Table X) is in accord with theory.

Reverting to the protein-acid system in the absence of salt, we may consider briefly the factor of protein concentration. Since

$$E_m=\frac{RT}{\mathbf{F}}\ln\left(1+\frac{z}{y}\right),$$

it is obvious that increasing z, while keeping y constant, will produce a rise in the potential. These conditions are fulfilled by increasing the concentration of the protein without changing the pH. Loeb (34) has investigated this question and here also his data confirm the theory.

The fact that his experimental results (see later for other investigations) could be explained fairly quantitatively by the Donnan theory led Loeb to the conclusion that proteins do not adsorb ions but must enter into simple chemical combination with them. While there may be reason for believing that the chemical view is correc it should be realised that the Donnan theory appl equally well on either assumption and affords no criterion for deciding the matter. It is, howeve tomary and convenient to regard the proteins as ionisable salts with acids and bases, rather than

complexes, and this standpoint is adopted in the following pages.*

Use has been made of membrane equilibria to investigate the state of protein salts in solution. Sporing (46) has studied the case of sodium caseinate by determining the distribution of sodium chloride in the presence of this substance. Analyses of the equilibrium solutions showed that $(m_{Na})_1$, the total concentration of sodium in the protein solution, was considerably greater than $\frac{(m_{Na})_2(m_{Cl})_2}{(m_{Cl})_1}$. The distribution equation gives

$$(f_{Na}m_{Na})_1 = \frac{(f_{Na}m_{Na})_2(f_{Cl}m_{Cl})_2}{(f_{Cl}m_{Cl})_1}.$$

Assuming that $(f_{Cl})_1 = (f_{Cl})_2$, it follows that $(f_{Na})_1$ must be much less than $(f_{Na})_2$. The low value for the activity coefficient of the sodium in the presence of the protein may be considered as due either to strong interionic action between the sodium and caseinate ions or to the formation of un-ionised caseinate.† Sporing adopts the latter alternative and calculates the degree of ionisation of the sodium caseinate on the assumptions that the activities of the diffusible ions are equal to their concentrations and that the sodium chloride is completely ionised. His values vary from 0·6 to 0·8. Wright (47), as the result of an investigation similar to that of Sporing,

* Rinde (26) has shown that Langmuir's adsorption isotherm is capable of representing satisfactorily Loeb's data for the combination of hydrogen ions with gelatin. Rideal (42), however, points out that the imple law of molecular proportions, as applied to the neutralisation of a ak base by a strong acid, may be put in a form which is substantially of the isotherm. Jordan-Lloyd (32) questions whether it is feasible erentiate sharply between chemical compounds and adsorption es in the case of such a large and complicated system as a protein. should also be made to the statements of McBain (43), Wilson (44), Lewis (31), and Hitchcock (45).

is (31).

obtains an average value of 0·68 for sodium caseinate and of 0·30 for the calcium salt. It is interesting to note that Pauli (48) deduces the range 0·62 to 0·67 for the sodium compound, from measurements of conductivity.

Northrop and Kunitz (49) have shown how membrane potential measurements may be employed to investigate the interaction between proteins and neutral salts. For the purpose of illustrating their procedure we may consider a solution containing gelatin and silver nitrate. If such a solution be allowed to come into equilibrium, across a membrane, with a pure solution of $AgNO_3$, then

$$m_c = m_t - m_i,$$

where m_t = total concentration of silver in the protein solution, m_c = concentration of silver combined with protein, and m_i = concentration of free silver. Also

$$E_m = \frac{RT}{n\mathbf{F}} \ln \lambda,$$

and

$$\lambda = \frac{f_0 m_0}{f_i m_i};$$

where m_0 = concentration of silver in the solution free from protein. Hence

$$m_c = m_t - \frac{\lambda f_0 m_0}{f_i}.$$

Since m_t and m_0 can be found by analysis of the equilibrium solutions, and λ is given by the membrane potential, we may ascertain the concentration of combined silver provided some reasonable assumption is made with regard to f_0/f_i, the ratio of the activity coefficients of the silver ion.* Northrop and Kunitz

* The experiments give directly the decrease in the activity of the silver ion in the presence of protein, but this decrease may be due to inter-ionic action as well as to combination with the protein. Some

assume that the effect of the protein on the ionic strength may be neglected, and suggest that, since in their experiments the total concentration of diffusible ions is much the same in the two solutions, f_0 be considered equal to f_i. Although the protein is present in small concentration, the assumption that its contribution to the ionic strength of the solution is negligible appears improbable at first sight, since the proteins are undoubtedly polyvalent. According to Simms (50), however, the effective valence of polyvalent ions decreases as the distance between the groups increases, and gelatin in acid solution may be regarded as possessing an effective valence of only 1·8.

The combination of protein with salt will, in general, increase the proportion of ionised protein, and, since the concentration of this component may be deduced from membrane equilibrium data, we have an alternative method of investigating the interaction between salts and proteins. Thimann (51) has calculated the effect of sodium chloride on the ionisation of gelatin from the results of some experiments by Loeb (37) and Loeb and Kunitz (52) on the systems:

z	G^+			z'	G^+		
y	H^+	H^+	x and	y'	H^+	H^+	x'
$z+y$	Cl^-	Cl^-	x	n	Na^+	Na^+	m
				$z'+y'+n$	Cl^-	Cl^-	$x'+m$
(1)		(2)		(1)		(2)	

The experiments give the membrane potentials and the activities of the hydrogen ion in the protein free solutions.

assumption with regard to the extent of the first of these effects is therefore necessary in order to calculate the decrease in the concentration of free ions. The symbol f_i refers to the free ions in the protein solution and not to the total silver.

Since the concentrations of salt in the latter are also known, it is evident that we have the material for calculating the concentrations of all the ions in the protein solutions. For the one system we have

$$z=[Cl^-]_1-[H^+]_1,$$

and for the other

$$z'=[Cl^-]_1-[H^+]_1-[Na^+]_1.$$

Any difference between z and z' when $y=y'$, and the total concentration of the protein is the same, must be due to combination of the protein with sodium ion. Table XI shows that such combination may be considerable.

TABLE XI.—COMBINATION OF GELATIN WITH SALTS

Concentration of Salt	Ion concentrations in milli-equivalents per litre				z'/z
	y	n	z'	z	
M/16000	0·044	0·017	0·749	0·552	1·36
M/500	0·099	1·20	2·31	1·16	1·99
M/100	0·152	8·81	2·58	1·76	1·47
M/16	0·181	62·00	1·02	1·94	0·52
M/8	0·189	125·0	0·0	1·95	

Increase in the concentration of the protein ions tends to increase the membrane potential (see p. 65). The magnitude of this effect, however, will be small compared with the depressive influence of the sodium chloride, since the potential is proportional to $\ln\left(1+\frac{z'}{y'+n}\right)$ and, as Table XI shows, n increases much more rapidly than z'.

Osmotic Pressure of Protein Solutions

A rational interpretation of the osmotic pressure of protein solutions is not only of importance in elucidating the nature of these systems, but is also of great significance for the study of the distribution of water in animal and vegetable tissues. As will be clear from the following discussion, the Donnan theory of membrane equilibria is essentially involved in any satisfactory interpretation.

Loeb and co-workers have given much attention to the problem of the variation of the observed osmotic pressure with change in conditions, and we shall first consider the influence of pH, as investigated by Loeb. Treating the protein as an ampholyte, the charged units of which are either large multivalent ions or ionic micelles, the Donnan equilibrium for solutions on the acid side of the isoelectric point is represented by the following diagram:—

a	B		
b	B^{n+}	H^+	x
z	Cl^-	Cl^-	x
y	H^+		
y	Cl^-		
(1)		(2)	

The negative protein units are omitted in this scheme, since their concentration is already small at the isoelectric point and diminishes as the solution becomes more acid. It should also be noted that $b=z/n$ and $a=c-z/n$, where c=total concentration of protein, and further, that n is an average value, since the valency may be variable. If the membrane is arranged to act as an osmometer the *recorded* pressure will be given, on the simple theory of Van't Hoff, by

$$P_1=RT(a+b)+RT(2y+z-2x).$$

The second term on the right-hand side represents that part of the pressure due to the difference in total concentration of the diffusible ions.

According to the Donnan equation, $2y+z$ must be greater than $2x$. We also have

$$x^2=y(y+z),$$

that is

$$z=\frac{x^2-y^2}{y};$$

therefore

$$2y+z-2x=\frac{(x-y)^2}{y}.$$

Fig. 2 shows the experimental curve obtained by Loeb (37) for the osmotic pressure of 1 per cent. gelatin solutions containing hydrochloric acid, together with the curve for $RT\frac{(x-y)}{y}$. The values of x and y were determined by means of the hydrogen electrode. It will be seen that the two curves are very similar. They rise in parallel fashion from the isoelectric point (pH 4·7), a practically constant difference existing between them up to pH 3·2. Since over this range the observed pressure curve is higher than the other, it appears probable that the difference is due to the protein units themselves, and indicates that the value of $(a+b)$ is not negligible although the solution is dilute. In any case it is evident that the variation of the observed osmotic pressure is the result of the Donnan distribution of the hydrochloric acid, and that there is no need to postulate an alteration in the degree of dispersion of the protein in order to account for the principal features of the experimental curve.

The variation of the observed pressure may be deduced from Hitchcock's equations (see pp. 31, 35), provided a slight change in formulation be introduced to allow for

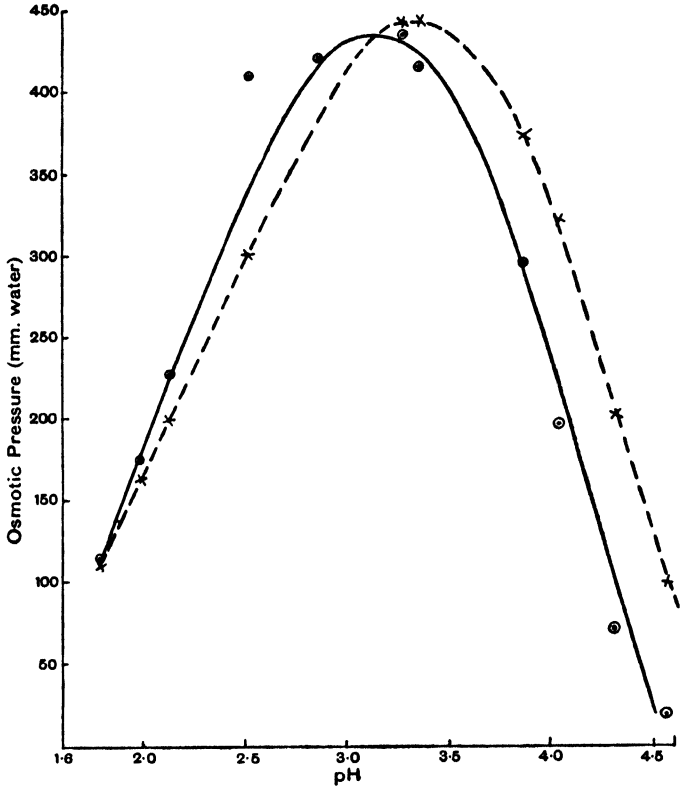


FIG. 2.—Observed and calculated osmotic pressures of 1 per cent. gelatin in hydrochloric acid solution. Abscissæ give the pH of the gelatin solution at equilibrium. Temperature = 24° C.

the polyvalency of the protein ions. Procter and Wilson (98) have suggested the following alternative method of approaching the question.* The equation of products can be put in the form

$$y^2+yz-x^2=0.$$

Hence

$$y=\frac{-z+\sqrt{4z^2+4x^2}}{2}.$$

* See also Donnan (8), Loeb (34), (35), (53).

By substitution in the osmotic pressure expression we obtain

$$P_1 = RTc + RT(-2x + \sqrt{4x^2 + 4z^2}).$$

When $x=0$, $z=0$, *i.e.* there is no excess of positive protein ions. With increasing values of x, z tends towards the limiting value nc, and at large values of x becomes negligible compared with x. Hence the second term of the pressure expression has the value 0 when x is 0 or ∞, and under these conditions $P_1 = RTc$. At some intermediate value of x, the second term, and hence P_1, must therefore pass through a maximum. The descending course of the curve for $RT(2y+z-2x)$ at low pH values is also to be expected from the discussion on p. 20, where it is shown that, if the concentration of the non-diffusible ion is kept constant, the observed osmotic pressure decreases as the concentration of the diffusible electrolyte is made larger.

According to Hitchcock (see p. 35), the approximate condition of maximum observed osmotic pressure is $pK = 2py - px$. Loeb's data for gelatin chloride show that the maximum was observed when $py = 3{\cdot}33$ and $px = 2{\cdot}87$. Hence $pK = 6{\cdot}66 - 2{\cdot}87 = 3{\cdot}79$. The data for maximum value of $(2y+z-2x)$ give a somewhat smaller value. Since the value of pK obtained from Hitchcock's (40) combination curve is 3·625, it may be said that Hitchcock's simplified theory applies fairly well.

Investigations leading to results similar to those for gelatin have been carried out by Loeb (54) on egg-albumin and casein and by Hitchcock (33), (55) on edestin and serum globulin. Loeb (56) and Loeb and Kunitz (41) have studied the effects of a considerable variety of acids on the osmotic pressure of gelatin solutions. These workers find that in general monobasic

acids give curves identical with that for HCl. On the other hand the pressures for sulphuric and sulphosalicylic acids lie on a common curve which, while of the same form as the HCl curve, shows a markedly lower maximum. When the anion of the acid is divalent the distribution equation becomes

$$y^2\left(\frac{y}{2}+\frac{z}{2}\right)=x^2\frac{x}{2},$$

or

$$z=\frac{x^3-y^3}{y^2}.$$

In the case of H_2SO_4, $\frac{x}{2}$=concentration of sulphate ions in the pure acid solution, $\frac{y}{2}$=concentration of sulphate ions of the free acid in the protein solution, and $\frac{z}{2}$=concentration of sulphate ions corresponding to the hydrogen ions combined with the gelatin. The calculated pressure difference due to the diffusible ions is given by $RT\left(\frac{3}{2}y+\frac{z}{2}-\frac{3}{2}x\right)$, which by substitution for z becomes $RT\left[\frac{(y-x)^2(2y+x)^2}{y^2}\right]$. The curve for the variation of this quantity with pH again closely resembles the curve of observed osmotic pressure (see Loeb (56)).

We may next consider the effect of adding the neutral salt MN to a gelatin-HCl system. It is evident from the general equation of products (equation 5) that $([H^+]+[M^-])\times([Cl^-]+[N^-])$ will have the same value in both solutions. If the concentration of the salt in the protein solution be denoted by v, and in the other by u, then

$$(x+u)^2=(y+v)(y+v+z).$$

The pressure difference due to the diffusible ions is equal to

$$RT[-2(x+u)+\sqrt{4(x+u)^2+z^2}]=P'.$$

It is obvious that, if the values of x and z are kept constant and the value of u is increased without limit, the value of P approaches zero as limit. In other words, the observed osmotic pressure of an acid solution of gelatin would decrease on the addition of a neutral salt, provided any combination between the latter and the protein is relatively small. It should be recognised that the value of the pressure would decrease, owing to the change in ionic distribution, quite apart from possible repression of the ionisation of the protein produced by the presence of a neutral salt. The experiments of Loeb (37) and Loeb and Kunitz (52) have provided ample evidence in support of this theory of the action of neutral salts. As may also be predicted, salts with divalent anions are more effective than those with monovalent anions, while the valency of the cation is not of importance.

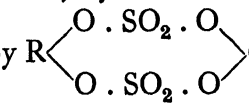
It will be evident that the effects of bases on the osmotic pressure of protein solutions may be treated in exactly the same way as those of acids. Here also experiment (Loeb (37), (34), Kunitz (57)) is in agreement with theory.

As previously indicated, the observed value of the osmotic pressure of a protein solution of concentration c is equal to the sum of the terms RTc and P', where P' depends upon the unequal distribution of the diffusible ions. Hence P' enters as a correction factor into the calculation of the molecular weight of the protein from the observed pressure. The complication due to the Donnan equilibrium was, of course, not realised by the

earliest investigators of the osmotic pressures of protein solutions at varying hydrogen-ion concentration (see, for example, Lillie (58)) and erroneous conclusions were drawn accordingly. At the isoelectric point of the protein P' becomes negligible, and the observed pressure is equal to that of the protein itself. P' may also be made very small by adding a large excess of electrolyte. This is the principle employed by Sørensen (59) in calculating the molecular weight of egg-albumin from the osmotic pressure of solutions of this protein in the presence of ammonium sulphate.

The simple osmotic equations proposed by Donnan and applied by Loeb naturally represent only approximately the behaviour of actual systems. Reference should be made to the papers of Adair (60), Adair and Callow (61), and Hückel (10) for more exact treatment of the subject.

Carrageen Extract

Purely chemical evidence suggests that the constitution of the complex mucilaginous substance extracted from carrageen (Irish moss) by treatment with cold water may be represented by  . To test this view Harwood (62) has examined the influence of the substance upon the distribution of calcium chloride across a membrane of parchment paper. The chloride became more concentrated on the side (2) of the membrane free from carrageen extract, and the equation

$$[Ca^{++}]_1/[Ca^{++}]_2=[Cl^-]_2^2/[Cl^-]_1^2$$

was found to hold if certain simple assumptions were made. No sulphate could be detected in solution (2). Harwood's experimental results support the conclusion

that the extract is a calcium salt of a sulphuric ester which resembles calcium chloride with regard to ionic behaviour.

Sodium Thymonucleate

In the course of an investigation into the condition in solution of the salts of thymonucleic acid, Hammarsten (63) has studied the membrane equilibria set up by these substances.

We may consider, for example, the distribution across a membrane of sodium chloride in the presence of sodium thymonucleate. The equilibrium state may be represented by

$$\begin{matrix}[Na^+]_T & [T^{\equiv}] \\ [Na^+]_1 & [Cl^-]_1\end{matrix} \;\Big|\; \begin{matrix}[Na^+]_2 \\ [Cl^-]_2.\end{matrix}$$

At equilibrium

$$([Na^+]_T+[Na^+]_1)[Cl^-]_1=[Na^+]_2[Cl^-]_2.$$

If the concentration of Na_4T is denoted by c, and we assume complete ionisation, then

$$(4c+[Cl]_1)[Cl]_1=[Cl]_2^2.$$

Hammarsten found that analysis of the actual equilibrium solutions gave approximately

$$(0{\cdot}8c+[Cl]_1)[Cl]_1=[Cl]_2^2.$$

Moreover, the observed osmotic pressure could be fairly well represented (up to a salt concentration of 0·002 M) by $RT(0{\cdot}8c+2[Cl]_1-2[Cl]_2)$. These results indicate that the degree of ionisation of sodium thymonucleate has a value in the neighbourhood of 0·2 under the conditions of Hammarsten's experiments, or, in more general terms, that the thymonucleate ion influences the activity of the sodium ion in a very marked manner.

Liquid Membrane

The necessary condition of constraint for the establishment of a Donnan equilibrium will be satisfied if a solution of one electrolyte be separated from a solution of a second by a layer of immiscible liquid which dissolves one of the electrolytes but not the other. Donnan and Garner (7) attempted to determine the distribution of lithium chloride in the system

KCl + LiCl in water (1)	Amyl alcohol	LiCl in water (2)

where LiCl is readily soluble in the liquid membrane, while KCl is practically insoluble. The diffusion of the lithium chloride through the alcohol was, however, found to be too slow for the required purpose, and the problem was therefore approached in an indirect manner.

Determinations were made of the distribution of lithium chloride between water and amyl alcohol in the presence and in the absence of potassium chloride. It will be seen that if a mixed aqueous solution and a pure aqueous solution of LiCl give rise to the same concentration of this salt in the alcohol, then these solutions would be in equilibrium if separated by a layer of the alcohol. The data obtained by Donnan and Garner are given in Table XII.

The ionic "concentrations" in the solutions were calculated by means of the degrees of ionisation for lithium chloride deduced by Green (*J.C.S.*, **93** (1908), 2023) from conductivity data.

Murray (64) has investigated the potential difference across a membrane of amyl alcohol separating solutions containing equal concentrations of lactic acid but

unequal concentrations of sodium lactate. The variation in the potential with increase in concentration of sodium lactate suggests that the Donnan potential may be treated as a special case of a diffusion potential.

TABLE XII.—DISTRIBUTION AT AN AMYL ALCOHOL MEMBRANE

KCl (1)	LiCl (1)	LiCl (2)	Undissociated LiCl		$[Li^+]\times[Cl^-]$	
			(1)	(2)	(1)	(2)
0·944	3·504	3·78	1·44	1·36	5·94	5·86
1·200	2·613	2·95	0·945	0·976	4·045	3·900
0·962	5·45	5·80	2·39	2·46	11·02	11·17

CHAPTER III

BIOLOGICAL AND TECHNICAL APPLICATIONS

FROM the first Donnan stressed the probability that ionic distribution equilibria of the kind described in the preceding pages would prove to be of importance in the investigation of biological problems. The outer surface of the protoplasm of the living cell acts as a semi-permeable membrane, which may be impermeable to certain inorganic ions or to the colloidal protein constituents of the cells and cavities of vegetable and animal organisms. This state of affairs might be expected to give rise to unequal distribution of the freely diffusible ions present, and to the concomitant osmotic and electrical conditions.

The researches of Loeb, Procter, and Wilson on the proteins *in vitro* undoubtedly stimulated interest in the biological aspect of the Donnan theory. While many points have yet to be made plain, the existence of Donnan equilibria *in vivo* would seem now to be definitely established, so that a knowledge of the principles underlying such equilibria has become an essential tool in the equipment of the cellular physiologist.

Due to the initiative of Procter, these principles have also contributed materially to an understanding of industrial processes which involve the swelling of certain plant and animal products.

EQUILIBRIA IN THE BLOOD

Distribution of Electrolytes between Cells and Serum.— The most striking application of Donnan's theory in the biological sphere relates to the distribution of diffusible

ions between the red blood corpuscles and the serum of the blood. That the concentrations of chloride and bicarbonate are not necessarily the same in these two constituents of the blood has been known for a considerable time, as has also the fact that an increase in the supply of carbon dioxide to the blood results in a transfer of chloride from serum to cells. A real insight into the mechanism underlying these and related phenomena has, however, been gained only within recent years, as the result of the researches initiated by Henderson (65), Warburg (66), and Van Slyke (67). Of particular importance is a paper by Van Slyke, Wu, and McLean (68), in which is presented a successful quantitative interpretation, based largely upon the principles elucidated by Donnan.

As simplified in Van Slyke's treatment the equilibrium between cells and serum may be represented by the following scheme:—

Cells			Serum		
$\left.\begin{matrix}\mathrm{Hb}\\ \mathrm{Hb^-}\\ \mathrm{Cl^-}\\ \mathrm{HCO_3^-}\end{matrix}\right\}$	$\mathrm{B^+}$	$\left\{\begin{matrix}a\\ b\\ c\end{matrix}\right.$	$\left.\begin{matrix}x\\ y\\ z\end{matrix}\right\}$	$\mathrm{B^+}$	$\left\{\begin{matrix}\mathrm{P}\\ \mathrm{P^-}\\ \mathrm{Cl^-}\\ \mathrm{HCO_3^-}\end{matrix}\right.$
(1)			(2)		

In this, B^+ represents the sum of sodium and potassium ions, Hb the hæmoglobin, and P the serum proteins. The complete scheme should include the cell proteins other than Hb, and the ions H^+, OH^-, Mg^{++}, Ca^{++}, $SO_4^{=}$, and $HPO_4^{=}$, but all these are present in such relatively small concentration that they may be neglected without introducing serious error. Now on the alkaline side of its isoelectric point (pH=6·8) hæmoglobin behaves as an acid and hence forms anions. Since these cannot pass through the cell membrane, the concen-

tration of every diffusible anion will be greater in (2) than in (1) when equilibrium is established. Moreover, according to the simplest form of the Donnan equation the distribution will be such that

$$\frac{[Cl^-]_1}{[Cl^-]_2}=\frac{[HCO_3^-]_1}{[HCO_3^-]_2}=\frac{[Cl^-]_1+[HCO_3^-]_1}{[Cl^-]_2+[HCO_3^-]_2}=r.$$

TABLE XIII.—DISTRIBUTION OF ELECTROLYTES IN BLOOD SATURATED WITH OXYGEN AT 38° C.

Chloride		Bicarbonate		$\frac{[Cl^-]_1}{[Cl^-]_2}$	$\frac{[HCO_3^-]_1}{[HCO_3^-]_2}$
(1)	(2)	(1)	(2)		
64·2	109·1	13·64	21·52	0·589	0·634
79·5	104·3	25·84	33·72	0·762	0·767
68·1	118·2	(12·49)	21·67	0·576	..
78·8	114·2	19·50	28·78	0·690	0·678
81·8	113·1	23·72	32·34	0·723	0·734
85·1	111·6	28·87	37·72	0·762	0·779
66·3	107·6	11·64	19·89	0·616	0·586
76·3	105·7	18·13	26·17	0·722	0·693
84·8	101·3	27·68	34·54	0·837	0·802
63·5	106·3	13·61	22·71	0·597	0·597
70·7	104·1	21·19	29·28	0·679	0·723
79·8	100·8	29·38	35·43	0·792	0·830

In Table XIII will be found the values obtained by Van Slyke, Wu, and McLean (68) for the chloride and bicarbonate contents of the cells and serum of normal horse blood at various carbon dioxide tensions. Concentrations are expressed as milliequivalents per kilogram of water, and the distribution ratios are calculated on the assumption of complete ionisation and equality of activity

coefficient in (1) and (2). The table shows quite definitely that the ratios vary in parallel fashion and are approximately equal in any given case.

Van Slyke, Wu, and McLean were able to express the ratio r in terms of the non-diffusible constituents (which include the sodium and potassium ions) of the cells and serum. They start from the equation

$$[B]_2+[Cl]_2+[HCO_3]_2=[B]_1+[Cl]_1+[HCO_3]_1+[HB],$$

which assumes equal osmotic activity in (1) and (2).* [Hb] represents the total concentration of hæmoglobin in units of oxygen combining capacity. The brackets indicate mols. per 1000 grams water. Expressing this equation in terms of concentrations of base by employing the algebraic symbols given in the diagram, we have

$$(x+y+z)+y+z=(a+b+c)+b+c+[Hb].$$

Hence

$$2(y+z)+x=2(b+c)+a+[Hb],$$

and

$$r=\frac{[Cl^-]_1+[HCO_3^-]_1}{[Cl^-]_2+[HCO_3^-]_2}=\frac{b+c}{y+z}=1-\frac{a+[Hb]-x}{2(y+z)};$$

or

$$r=1-\frac{a+[Hb]-x}{2([B]_2-x)} \quad . \quad . \quad . \quad . \quad . \quad (29)$$

Substituting for a and x by means of empiric equations based on the experiments of Van Slyke, Wu, and McLean (*loc. cit.*), and of Van Slyke, Hastings, Heidelberger, and Neill (69) with horse blood, we can express r in terms of the concentrations of hæmoglobin, serum protein, total serum base, the pH values of cells and serum, and the oxygenation. The actual distribution of chloride and bicarbonate in the case of horse blood in any given

* The osmotic activity of P is comparatively negligible.

condition is found to be very near to that predicted by the equation obtained as above.

This equation, however, does not enable one to predict the variation, for a given blood, of r with pH. Changes in pH and oxygen content bring about an alteration in the distribution of water between the cells and the serum, so that the concentrations of the non-diffusible constituents do not remain constant. By expressing concentrations in units of substance per kilo of *whole blood* the equation assumes the form,

$$r=1-\frac{a'+[\mathrm{Hb}]'}{2(b'+c')+[\mathrm{Hb}]'}-\frac{x'}{2(y'+z')},$$

where a', $[\mathrm{Hb}]'$, etc. = millimols of the respective substances per 1000 grams blood.

$$\text{Hence } r=1-\frac{a'+[\mathrm{Hb}]'}{2([\mathrm{B}]_1'-a')+[\mathrm{Hb}]'}-\frac{x'}{2([\mathrm{B}]_2'-x')} \quad . \qquad (30)$$

For any given blood $[\mathrm{Hb}]'$, $[\mathrm{B}]_1'$ and $[\mathrm{B}]_2'$ are constant. Also a' is a function of $[\mathrm{Hb}]'$, pH_1, and the oxygen content of the cells, and x' a function of $[\mathrm{P}]'$ (also a constant) and pH_2. Equation (30) therefore permits of the calculation of the variation of r with pH. Fig. 3, which is taken from the paper of Van Slyke, Wu, and McLean, shows that the distribution ratios obtained experimentally by these workers lie in the neighbourhood of the theoretical curve.

According to Donnan's equation,

$$\frac{[\mathrm{H}^+]_2}{[\mathrm{H}^+]_1}=r,$$

or

$$\begin{aligned}-\log r &= -\log[\mathrm{H}^+]_2+\log[\mathrm{H}^+]_1\\ &=\mathrm{pH}_2-\mathrm{pH}_1.\end{aligned}$$

Warburg (66) has estimated values of $\mathrm{pH}_2-\mathrm{pH}_1$ in

horse blood, and his maximum values agree well with the theoretical curve for $-\log r$ (Fig. 3).

In view of these results it appears that the mechanism of the changes in the blood, which accompany variation of the partial pressure of the carbon dioxide, is as follows.

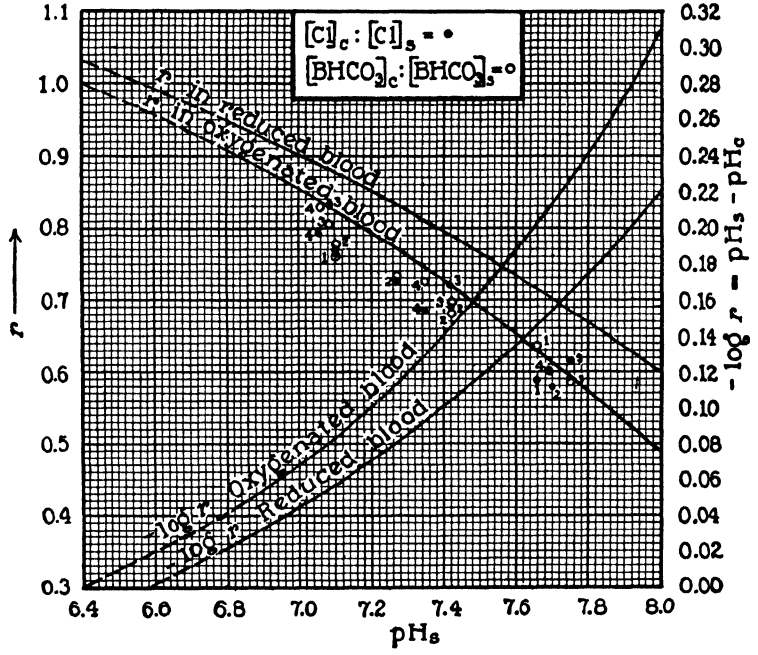


FIG. 3.—Observed and calculated variation of r for horse blood of average serum and cell composition

(From Van Slyke, Wu, and McLean, *Journal of Biological Chemistry*, 1923)

The cells are essentially a solution containing hæmoglobin—functioning as an acid—hydrochloric acid, and carbonic acid, in equilibrium with base. The serum is a similar system with hæmoglobin replaced by serum proteins. These are present in such small quantity that they may be disregarded in a first approximation. Owing to the buffer action of the hæmoglobin, increase

in the carbon dioxide tension (*i.e.* increase in the concentration of free H_2CO_3) leads to the formation of sodium bicarbonate at the expense of the hæmoglobin salts. Since the corresponding change in the serum is relatively negligible, the primary effect of the decrease in pH is to make $\frac{[HCO_3^-] \text{ in cells}}{[HCO_3^-] \text{ in serum}}$ greater than $\frac{[Cl^-] \text{ in cells}}{[Cl^-] \text{ in serum}}$. To restore equilibrium Cl^- must therefore migrate from cells to serum, exchanging with an equivalent amount of HCO_3^-, until the above ratios become equal. In addition water must pass from serum to cells to maintain osmotic equilibrium.

The above simple picture corresponds to the equation,

$$r = 1 - \frac{[Hb^-]}{([Cl^-]+[HCO_3^-])_{serum}},$$

or, in terms of the symbols previously used,

$$r = 1 - \frac{a}{2(y+z)} \quad . \quad . \quad . \quad (31)$$

(*cf.* equation (29).)

The equation shows that the greater the amount of base neutralised by hæmoglobin, the greater is the inequality in electrolyte distribution, and *vice versa*.

The effects of oxygenation and reduction may now be considered. At a given pH oxygenated hæmoglobin binds more base than reduced hæmoglobin, or, in other words, is more ionised. Hence, according to equation (31), the value of r must decrease on oxygenation, and chloride migrate from cells to serum, as was first observed by Henderson and McLean (65). Van Slyke, Hastings, Murray, and Sendroy (70) found that the differences in the value of r for oxygenated and reduced horse bloods, over the pH

range 7·0–7·6, approximate to those predicted from the equation of Van Slyke, Wu, and McLean.

Henderson and co-workers (71) obtained values of r for oxygenated and reduced normal human blood, at different carbon dioxide tensions, which agreed fairly closely with the values for horse blood under comparable conditions. The later and more extensive investigation of Hastings, Sendroy, McIntosh, and Van Slyke (72) on normal and pathological specimens of human venous blood has established, however, that the value of the distribution ratio in man is, on the average, several per cent. higher than in the case of the horse. This is in accord with theory, since Adair (73) has shown that the base binding power of human cells is less than that of horse cells. Actual calculation of r for human blood, from the equation of Van Slyke, Wu, and McLean, on the basis of Adair's analyses, gives values which approximate to those observed. Of considerable interest is the fact that the distribution in the pathological bloods examined by Hastings, Sendroy, McIntosh, and Van Slyke was not materially different from the distribution in normal blood.

The last-named workers found that the relation between the distribution ratios of chloride and of bicarbonate in human blood was in any given case,

$$\frac{[Cl^-] \text{ in cells}}{[Cl^-] \text{ in serum}} = 0{\cdot}87 \frac{[HCO_3^-] \text{ in cells}}{[HCO_3^-] \text{ in serum}}.$$

Van Slyke, Hastings, Murray, and Sendroy had previously obtained the factor 0·81 for horse blood. This inequality of the ratios, which forms the most striking discrepancy between fact and theory, should be considered in the light of the important research by Henriques, discussed in the following section (p. 89).

Hastings and Van Dyke (74) report that when bromide is added to blood, the value of $[Br^-]_{cells}/[Br^-]_{serum}$ is considerably higher than that of $[Cl^-]_{cells}/[Cl^-]_{serum}$, though the variation in the bromide ratio with change in pH and oxygenation is in accord with equation (29). The discrepancy is particularly marked in the blood of dogs fed with large quantities of sodium bromide. These results suggest that the relationships are more complicated when bromide is present.

Hastings, Harkins, and Leiter (75) have studied the distribution of chloride and bicarbonate in experimental acidosis and alkalosis. In general their data confirm the theory of Van Slyke.

The Properties of Hæmoglobin.—Chemical evidence suggests strongly that in the blood hæmoglobin functions as a weak acid. The Donnan equilibrium offers an independent and more direct means of investigating this question. If hæmoglobin is brought into membrane equilibrium with some diffusible electrolyte, the magnitude and sign of the potential difference across the membrane will show in what manner the hæmoglobin is acting. Taylor (76) has measured the electromotive force of the cell:

Cal. (satd.), KCl (satd.),	Laked corpuscles	Collodion membrane.	Dilute NaCl, + $NaHCO_3$	KCl (satd.), Cal. (satd.)
	(1)		(2)	

His results showed the presence of an appreciable membrane potential, and in every case the hæmoglobin solution was negative to the other. This means that the concentration of diffusible anions was greater in the crystalloid solution, a condition which can be established

only if the non-diffusible hæmoglobin is acting as an anion.

On bringing the hæmoglobin to the acid side of the isoelectric point, the sign of the membrane potential changed. This also indicates that the potential difference is the result of a Donnan equilibrium produced by the hæmoglobin.

A detailed investigation of the above system by Henriques (77) has yielded results of great interest and importance. Henriques found that at pH=6·6, the isoelectric point of salt-free hæmoglobin, a considerable membrane potential is set up, the sign of which shows that the hæmoglobin is negatively charged. The same behaviour is observed when dilute phosphate solution is substituted for the mixture of chloride and bicarbonate.

In Table XIV are given the data from one series of experiments with phosphate, the pH being varied by the addition of very small amounts of hydrochloric acid.

TABLE XIV.—MEMBRANE POTENTIALS IN HÆMOGLOBIN-PHOSPHATE SYSTEMS

Observed			"Calculated"	
* pH (1)	pH (2)	E_m millivolts	E_m millivolts	pH (1)
6·87	7·05	12·0	10·5	6·84
6·83	7·03	11·0	11·5	6·84
6·54	6·70	7·5	9·5	6·57
6·23	6·30	5·0	4·0	6·21
6·17	6·25	4·5	4·5	6·18
5·91	5·94	2·0	2·0	5·91
5·85	5·86	2·0	0·5	5·83
5·58	5·59	0·0	0·5	5·59

* *I.e.* $-\log a_H$.

The agreement between observed and "calculated" values of E_m and pH (1) shows that equilibrium was established in every case. Judging from the membrane potential, the hæmoglobin in this particular instance did not become electrically neutral until the pH was in the neighbourhood of 5·5. Similar results were obtained in two other series of experiments, and Henriques arrived at the conclusion that hæmoglobin must combine with phosphate ions to form negatively charged complexes.

A more elaborate study was made of the physiologically important hæmoglobin-($NaHCO_3 + NaCl$) systems, in order to determine the extent of complex formation. For this purpose, in addition to measurements of pH (1), pH (2), and E_m, the solutions (1) and (2) were analysed for chloride and total carbon dioxide content. The following shows the manner in which Henriques employs his data.

By the law of molecular proportions, in any solution of sodium bicarbonate,

$$\frac{1}{a_{H+}} = \frac{1}{K_1} \cdot \frac{a_{HCO_3^-}}{a_{CO_2}},$$

or

$$pH = pK_1 + \log \frac{y[HCO_3^-]}{z[CO_2]},$$

where y and z are the respective activity coefficients of bicarbonate ion and free carbon dioxide. Under the given conditions z may be taken as equal to unity without serious error. In this case

$$pH = (pK_1 + \log y) + \log \frac{[HCO_3^-]}{[CO_2]}.$$

At constant temperature and a given ionic strength we may therefore write:

$$pH = pK_1' + \log \frac{[HCO_3^-]}{[CO_2]},$$

or

$$pH = pK_1' + \log \frac{[HCO_3^-]}{[\text{Total } CO_2] - [HCO_3^-]}.$$

Hence

$$[HCO_3^-] = \frac{[\text{Total } CO_2]}{\text{antilog } (pK_1' - pH) + 1}.$$

According to Warburg (66) the value of pK_1 at 18° C. (the temperature of Henriques's experiments) is 6·51. Also Hastings and Sendroy (143) have shown that at 18° C.

$$\log y = -0{\cdot}50\sqrt{\mu},$$

where μ is the ionic strength referred to 1000 grams water.

Hence $$pK_1' \text{ (at } 18°) = pK_1 + \log y,$$

$$= 6{\cdot}51 - 0{\cdot}50\sqrt{\mu},$$

and

$$[HCO_3^-] = \frac{[\text{Total } CO_2]}{\text{antilog } (6{\cdot}51 - 0{\cdot}50\sqrt{\mu} - pH) + 1} \quad . \quad (31a)$$

In addition we have

$$a_{HCO_3^-} = y[HCO_3^-]$$

$$= (\text{antilog } -0{\cdot}50\sqrt{\mu})[HCO_3^-].$$

Equation (31*a*) enables us to calculate the activity of the bicarbonate ion in solution (2), since the experiments give the values of $[\text{Total } CO_2]_2$, pH (2), and μ_2. Knowing $(a_{HCO_3^-})_2$ we can obtain the value of $(a_{HCO_3^-})_1$ directly from the membrane potential by the usual equation,

$$E_m = \frac{RT}{\mathbf{F}} \ln \frac{(a_{HCO_3^-})_2}{(a_{HCO^-})_1}.$$ *

* *Cf.* Northrop and Kunitz (49).

The next step is to deduce the value of $[HCO_3^-]_1$ from $(a_{HCO_3^-})_1$. For this we need to know y. Henriques assumes in the first place that y_1 (the activity coefficient of the bicarbonate ion in (1)) is given by

$$y_1 = \text{antilog } -0{\cdot}50\sqrt{\mu_1},$$

and that the contribution of the hæmoglobin to the ionic strength is that of a monovalent anion. The value of [Total CO_2] in (1) may now be obtained from the expression,

$$[HCO_3^-]_1 = \frac{[\text{Total } CO_2]_1}{\text{antilog } (6{\cdot}51 - 0{\cdot}50\sqrt{\mu_1} - \text{pH})\ (1) + 1}.$$

(*Cf.* equation 31*a*.)

By [Total CO_2] is meant the sum of the concentrations of bicarbonate ion and of free dissolved carbon dioxide. Henriques found that the "Total" carbon dioxide calculated in the above manner fell considerably short of the carbon dioxide actually present in (1). The difference represents that part of the carbon dioxide which is combined with the hæmoglobin.

The concentration of free chloride ions in the hæmoglobin solution is given by

$$\log (f_{Cl})_1 [Cl^-]_1 = \log (f_{Cl})_2 [Cl^-]_2 - \frac{\mathbf{F}E_m}{RT}.$$

Experiment gives the values of $[Cl^-]_2$ and E_m, and $(f_{Cl})_1$ and $(f_{Cl})_2$ may be obtained by the use of the relation.

$$f_{Cl} = \text{antilog } -0{\cdot}35\sqrt{\mu}.$$

(Lewis and Randall (16).)

Henriques's data show that the total concentration of chloride in (1), as determined by analysis, is appreciably greater than the calculated concentration of free chloride ions; indicating combination with the hæmoglobin.

The alternative to Henriques's view is, of course, that while the bicarbonate and chloride ions do not combine with the hæmoglobin, their activity is greatly decreased by the presence of the protein. If, however, Henriques's figures are interpreted in this sense, it follows that the variation of the activity coefficient of the bicarbonate ions with change in pH is very different from that of the chloride ions. This appears very improbable. Moreover, as already indicated, and as Table XV illustrates, membrane potential measurements point directly to the formation of negatively charged complexes between the hæmoglobin and the carbon dioxide or chloride.

TABLE XV.—MEMBRANE POTENTIALS IN HÆMOGLOBIN ($NaHCO_3 + NaCl$) SYSTEMS

CO_2 pressure (mm. Hg)	E_m (milli-volts)	pH (2)	pH (1)	pH (1) (calcu-lated)
35	14·5	7·45	7·19	7·20
35	15·5	7·49	7·23	7·22
92	11·5	7·15	6·91	6·96
163	9·5	7·0	6·88	6·84
163	9·0	6·95	6·85	6·79
163	10·0	7·0	6·80	6·83
305	7·5	6·77	..	6·73
305	7·5	6·70	..	6·53
591	5·0	6·38	..	6·29

As was mentioned on p. 87, the distribution ratios of bicarbonate and chloride between red blood corpuscles and serum are not equal. It would appear from the work of Henriques that complex formation must account for part at least of this divergence from the simple theory of Van Slyke.

State of Calcium in the Blood.—If blood is dialysed against an aqueous solution, calcium is found in the dialysate. R. F. Loeb (78) has investigated the distribution of calcium chloride in membrane equilibrium with blood serum. The behaviour of this system was found to be very similar to that of simple protein systems. On the alkaline side of the isoelectric point, the concentration of calcium in the serum solution was greater than in the other, conditions being reversed on the acid side. Also the ratio $[Ca]_{serum}/[Ca]_{aqueous}$ varied directly with the concentration of serum protein. These facts show that the Donnan equilibrium is one of the chief factors controlling the "diffusibility" of the blood calcium.

If all the calcium and chloride are present in the form of free ions and interionic action is absent, then $\sqrt{[Ca]_{serum}}/\sqrt{[Ca]_{aqueous}}$ will be equal to $[Cl]_{aqueous}/[Cl]_{serum}$. Loeb found that in alkaline solution the first of the ratios was always higher than the second. Moreover, further investigation (79) showed that as the concentration of calcium chloride (or sodium chloride) was decreased, the inequality in the distribution of the calcium increased, while the chloride ratio remained constant. Loeb and Nichols (80) have shown by experiments on serum extracted with ether that the serum lipoids are not responsible for the divergences from the simple theory. They conclude, therefore, that the calcium probably forms a complex ion with the serum proteins.*

The Intraocular Fluids

The Aqueous Humour.—Various theories have been advanced to account for the origin of the fluids occurring in the cavities of the eye. For example, it has been held by

* *Cf.* Nitschke (81), Nitschke and Freyschmidt (82).

many that the aqueous humour (which occupies the space between the lens and the front of the eye) is the result of special secretory activity, whilst others regard it as being formed from the blood by simple transudation. The recent researches of Duke-Elder (83), (84), however, suggest very strongly that this fluid is a dialysate from the blood plasma of the capillaries which surround the eye.

Table XVI shows the main results * of analyses carried out by Duke-Elder (83), (85) on the aqueous humour and the blood serum of the horse.

TABLE XVI.—COMPOSITION OF SERUM, AQUEOUS HUMOUR, AND VITREOUS BODY †

	Serum	Aqueous	Vitreous
Total protein .	7·3692	0·0201	0·0652
Fats . .	0·13	0·004	0·007
Non-protein nitrogen .	0·0239	0·0236	0·0264
Sugar . .	0·0910	0·0983	0·0973
Sodium . .	0·3351 (146)	0·2787 (121)	0·2731
Potassium .	0·0201 (5·1)	0·0189 (4·8)	0·0192
Calcium . .	0·0101 (2·5)	0·0062 (1·5)	0·0068
Magnesium .	0·0028 (1·2)	0·0026 (1·1)	0·0020
Chlorine . .	0·3664 (103)	0·4371 (123)	0·4168
P (inorganic) .	0·0030 (PO_4=1·26)	0·0033 (PO_4=1·38)	0·0031
S („) .	0·0058 (SO_4=1·7)	0·0061 (SO_4=1·8)	0·0062

It will be seen that only traces of proteins are present in the aqueous; which is to be ascribed to the inability of colloidal material to pass through the walls of the

* Detailed analysis revealed that all the constituents of the serum were to be found in the aqueous.

† Values within brackets=millimols per litre. In other cases the concentrations are expressed as grams per 100 c.c.

capillaries. On the other hand, the diffusion of the sugar, a crystalloidal non-electrolyte, is not affected by these membranes, and hence this substance has substantially the same concentration in both aqueous and serum. The relative concentrations of the inorganic ions, however, afford the most striking evidence in favour of the view that the aqueous is derived by dialysis of the blood. On this view we should expect that a Donnan equilibrium would be set up between the two sides of the capillary membrane, and the experimental observations show that in all probability this is what actually happens.

The inorganic ions are seen to be unequally distributed, the concentration of the cations being greater in the serum than in the aqueous, and the opposite holding in the case of the anions. Since the sodium, calcium, etc., are associated with the protein to a certain extent, while the chloride is quite free, the unequal distribution of the latter receives a natural interpretation in terms of a Donnan equilibrium. For example, we may represent the distribution of sodium chloride by

Capillary Blood	Aqueous Humour
$Na^+\begin{cases}A^-\\Cl^-\end{cases}$	$Na^+ \quad Cl^-$

(A = protein.)

The inequality in distribution of the phosphate and the sulphate is not so pronounced as in the case of the chloride. Duke-Elder applies the Donnan equation to his figures for sodium and chloride. Thus

$$[Na^+]_{Aq}[Cl^-]_{Aq} = [Na^+]_{Blood}[Cl^-]_{Blood},$$
$$121 \times 123 = 146 \times 103,$$
$$149 = 150$$

The chloride content of the venous plasma is of course

lower than that of the arterial plasma. Analyses by Duke-Elder (83), (85) of the plasma of the rabbit gave the following values:—

	Chloride as grams NaCl per 100 grams water	
Arterial plasma .	0·619	0·6482
Venous plasma .	0·589	0·6213

It appears probable therefore that the concentration of chloride in the capillaries will not be the same as in the serum. Since, however, any correction (of the ionic product given above) in this respect apparently will not amount to more than a few per cent., we may say that the Donnan expression holds for the distribution of sodium chloride between the aqueous humour and the capillary blood of the eye.

The Vitreous Body.—As the result of investigations on the vitreous body, which fills the anterior chamber of the eye, Duke-Elder (86), (84), (87) concludes that it is a simple gel, percolated by a liquid closely resembling the aqueous humour. Chemical analysis of the vitreous in the horse shows that it contains all the constituents of the aqueous in comparable proportions, except for the protein, which is definitely higher (see Table XIV). The difference in protein content is due to the presence of two special substances, which appear to be responsible for the transparency of the gel structure of the vitreous.

It is evident that the same arguments as were used in the case of the aqueous may be advanced in support of the view that the bulk of the vitreous is a dialysate of the capillary blood. Further evidence in the same direction is supplied by some experiments of Duke-Elder (87) in

which the effect of paracentesis of the vitreous chamber on the composition of its contents was studied. The experiments were performed on rabbits, the intra-ocular pressure of one eye being lowered suddenly by a paracentesis in which 0·25 c.c. of vitreous was removed, and the amounts of protein, chloride, and sugar present in the abnormal vitreous determined at various times after paracentesis. Parallel determinations of the composition of the normal vitreous, from the other, untouched, eye, were made for comparison. In all cases the estimations were carried out on the filtrate of the whole vitreous body. The final results are given in Table XVII and Fig. 4.

TABLE XVII.—EFFECT OF PARACENTESIS ON THE DISTRIBUTION OF PROTEIN AND CHLORIDE

Time after paracentesis	Change in grams per 100 c.c. fluid	
	Protein (abnormal-normal)	Chloride (normal-abnormal)
1 hour	0·73	0·050
2 hours	2·37	0·118
6 „	1·47	0·103
48 „	0·88	0·069
6 days	0·125	0·040

These observations can be explained as follows. As the result of the decrease of intraocular pressure, the walls of the capillaries dilate and become more permeable to the proteins, which therefore appear in the vitreous to a greater extent than is normally the case. The original Donnan equilibrium is thus upset and a new one established by the passage of chloride ions from the vitreous fluid to the capillary blood. About two hours

after the puncture the vitreous begins to return to its usual state, which entails a decrease in the protein and a corresponding increase in the chloride. As is to be expected, the sugar undergoes practically no change throughout. Paracentesis also causes a shift in the protein and chloride of the aqueous similar to the above, and it is significant that if the operation is performed in

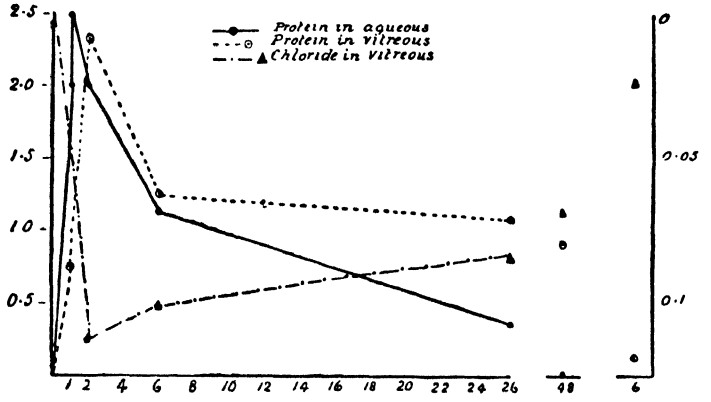


FIG. 4.—Percentage of protein and chloride, in aqueous or vitreous, as a function of the time after paracentesis
(From Duke-Elder, *Journal of Physiology*, 1929)

the presence of adrenaline or cocaine, which oppose dilation of the capillaries, the changes are not so marked as in the absence of such substances.

In conclusion it should be added that the results of the investigations of Duke-Elder on the osmotic pressure of the intraocular fluids (83) and on the influence of drugs on the intraocular pressure (88) are compatible with the theory of membrane equilibrium discussed above. The same may also be said with regard to the changes in the electrical potential difference between the aqueous and the blood observed by Lehmann and Meesmann (89).

Other Body Fluids

Relations similar to those between the blood plasma and the intraocular fluids are found in the cases of other body fluids. Data from an investigation by Hastings, Salvesen, Sendroy, jun., and Van Slyke (90) on œdema fluid, which was almost free from proteins, are given in Table XVIII.

Table XVIII.—Distribution between Serum and Œdema Fluid

	Serum	Fluid	r
HCO_3^-	29·3	30·4	0·964
Cl^-	116·8	120·0	0·972
Na^+	166·8	156·2	0·937

The distribution in this instance would appear to result from the establishment of a simple Donnan equilibrium.

Similar values for the distribution of the chloride have been obtained by R. Loeb, Atchley, and Palmer (91), Gollwitzer-Meier (92), and Michaud (93). The most marked deviation from the simple theory occurs in the distribution of potassium. For example, R. Loeb and co-workers (*loc. cit.*) obtain the value 0·62, and Gollwitzer-Meier (*loc. cit.*) the value 0·71 for the ratio $[K]_{fluid}/[K]_{serum}$.

The protein content of cerebro-spinal fluid is higher than that of œdema fluids. According to theory the inequality in the ionic distribution should therefore be less marked. Observation shows this to be the case (Mestrezat (94), Michaud (*loc. cit.*)).

Since the inorganic cations are not restricted in their

movement in the systems at present under consideration, we may expect the osmotic pressure of the serum to be greater than that of the œdema fluid. Water will therefore tend to be drawn into the serum, and hence some counter force must operate in order to produce a flow of œdema liquid or of lymph from the blood into the inter-cellular spaces.

Ionic Nature of Enzymes

Northrop (95) has drawn attention to the following interesting application of the principle of the Donnan equilibrium. If the distribution of the diffusible substance M in membrane equilibrium is such that

$$\frac{(M)_2^{\frac{1}{q}}}{(M)_1^{\frac{1}{q}}} \quad \text{or} \quad \frac{(M)_1^{\frac{1}{q}}}{(M)_2^{\frac{1}{q}}} = \frac{(N^{p+})_2^{\frac{1}{p}}}{(N^{p+})_1^{\frac{1}{p}}},$$

where N is an ion of known valency, then M must be a cation or anion, as the case may be, of valency q. Thus, in order to determine if a given diffusible substance is ionic in nature we may set up a membrane equilibrium and compare the distribution ratio with that of some ion such as H^+ or Cl^-. Equality of the ratios would seem to justify the conclusion that the substance in question is present in solution in the ionic condition. On the other hand, if the ratios are unequal no definite conclusion can be drawn.

The method was used by Northrop to investigate the properties of the enzymes trypsin and pepsin. Particles of gelatin were immersed in a solution containing the enzyme and chloride ions,* and the equilibrium distribution ratios determined for various pH values. Some of Northrop's data for trypsin are given in Table XIX and

* See following section for discussion of distribution equilibria in the system gel-aqueous solution.

suffice to show that, except in the neighbourhood of the isoelectric point of the gelatin, the ratios were practically equal over the pH range 2·0–11·0.

TABLE XIX.—DISTRIBUTION OF CHLORIDE AND TRYPSIN BETWEEN GEL AND SOLUTION

pH	$\frac{\text{Cl in liquid}}{\text{Cl in gelatin}}$	$\frac{\text{Trypsin in gelatin}}{\text{Trypsin in liquid}}$
2·0	0·60	0·50
2·5	0·40	0·30
3·0	0·24	0·23
4·7	1·0	?
5·5	1·3	?
6·0	1·6	1·4
7·0	1·9	1·9
8·0	1·9	1·7
9·0	2·0	2·0
10·0	2·3	1·9
10·6	0·50	0·43
11·0	0·45	0·30

Northrop also tried the effect of increasing concentrations of salt upon the equilibrium. The results for sodium nitrate are shown in Table XX. In this case the pH was 3·5.

TABLE XX.—INFLUENCE OF SALT CONCENTRATION ON THE DISTRIBUTION OF TRYPSIN

Concentration of salt	$\frac{\text{Cl in liquid}}{\text{Cl in gelatin}}$	$\frac{\text{Trypsin in gelatin}}{\text{Trypsin in liquid}}$
0	0·14	0·13
0·02	0·25	0·34
0·08	0·41	0·39
0·16	0·50	0·65

The experiments show definitely that the distribution of the trypsin between the gelatin particles and the surrounding solution is regulated by the same forces as are involved in the distribution of the chloride ions. It would therefore appear that trypsin is a diffusible monovalent cation from pH=2 to pH=10·2, and becomes a negative ion in more strongly alkaline solutions. Presumably the isoelectric point lies in the region of pH=10·2.

Similar results were obtained with pepsin, which was found to behave as a monovalent anion over the pH range 1 to 7.

The Swelling of Gels

Gelatin.—While isoelectric gelatin swells considerably when in equilibrium with water alone, it can be made to swell to a very much greater extent by immersion in a solution of acid or alkali of suitable concentration. For example, Jordan-Lloyd (32) states that leaf gelatin undergoes a sevenfold increase of volume in water, but that in HCl of pH=2·3 there is an additional increase of 33 volumes. Investigation of the relation between amount of swelling and concentration of HCl shows that with increasing concentration the swelling attains a maximum and then diminishes. Procter (96) was the first to suggest that the effect of the acid might be explained on the basis of a Donnan equilibrium. He postulated that the gelatin and acid interacted to give ionisable salts and that the colloidal gelatin ions so produced were unable to diffuse out of the gel. Since the gel was freely permeable to the H and Cl ions present, it followed that these must be distributed between the gel and the surrounding solution in accordance with the Donnan equation of ionic products. Procter thought that such distribution

might cause an excess of osmotic pressure in the jelly, which would lead to entry of water into the latter and hence swelling.

In order to test his views, Procter carried out an extensive series of experiments in the following manner. One gram (dry weight) portions of thin purified bone gelatin were placed in separate stoppered bottles, each of which contained 100 c.c. of HCl of known strength (c). After soaking for 48 hours, which proved sufficient for the establishment of equilibrium, each piece of gelatin was filtered off from the remaining liquid, which was titrated with alkali. This gave the concentration (x) of the external acid at equilibrium. The volume of external solution was also measured and subtraction of its value from 100 c.c. gave the volume of solution absorbed by the jelly. As a check the gel was weighed. Since the volume of the external solution and the difference between c and x were known, the total amount of acid absorbed by the gelatin could be determined. The gelatin was returned to the bottle and enough sodium chloride added to give an approximately saturated solution with the water held by the swollen jelly. As the result of this treatment the gel contracted and when equilibrium was reached (about 24 hours) had given up most of its absorbed solution and become a thin, horny plate. Titration of the expelled acid gave the concentration (y) of the free acid present in the jelly.* The amount of acid combined with the gelatin was obviously equal to the difference between the total acid absorbed and the free acid.

Assuming complete ionisation of the gelatin salt and

* The concentration of the small amount (about 1·5 c.c.) of free acid which remained in the dehydrated gel was assumed to be the same as that of the expelled acid.

denoting the concentration of gelatin ion (*i.e.* of combined H^+) by z, we have at equilibrium

z	GH^+		
y	H^+	H^+	x
$y+z$	Cl^-	Cl^-	x
Jelly		External solution	

TABLE XXI.—DISTRIBUTION OF HYDROCHLORIC ACID BETWEEN GEL AND EXTERNAL SOLUTION

c	Weight solution absorbed by gelatin	x	y	$\frac{x^2}{y}$	Concentration total acid absorbed by gelatin*
0·300	19·98	0·2950	0·2603	0·334	0·332
0·250	20·22	0·2450	0·2104	0·285	0·281
0·200	22·10	0·1945	0·1636	0·232	0·226
0·200	22·68	0·1940	0·1645	0·228	0·225
0·200	20·59	0·1925	0·1612	0·230	0·229
0·175	23·48	0·1685	0·1383	0·205	0·200
0·150	24·24	0·1435	0·1182	0·174	0·172
0·150	24·00	0·1434	0·1180	0·175	0·173
0·125	24·36	0·1180	0·0897	0·147	0·148
..	29·75	0·1052	0·0847	0·131	0·128
0·100	26·38	0·0944	0·0723	0·123	0·121
0·100	23·09	0·0930	0·0716	0·121	0·126
0·075	29·12	0·0680	0·0534	0·087	0·092
0·075	27·85	0·0666	0·0499	0·089	0·095
..	34·01	0·0576	0·0433	0·077	0·079
0·050	31·07	0·0420	0·0298	0·059	0·068
0·050	36·42	0·0406	0·0299	0·055	0·061
0·025	48·13	0·0172	0·0091	0·033	0·031
0·025	40·44	0·0170	0·0093	0·031	0·037
0·020	51·72	0·0122	0·0061	0·025	0·027
0·015	51·89	0·0120	0·0062	0·023	0·027
0·015	52·20	0·0077	0·0018	0·033	0·022
0·015	57·91	0·0073	0·0022	0·024	0·020
0·010	53·68	0·0032	0·0005	0·021	0·017
0·010	58·43	0·0028	0·0004	0·020	0·015
0·010	59·90	0·0025	0·0004	0·016	0·015
0·008	48·70	0·0018	0·0004	0·008	0·015
0·006	44·11	0·0011	0·0005	0·003	0·014

* On the assumption that the gelatin salt is completely ionised, the concentration of Cl ion in the gel will be equal to the total acid.

Table XXI shows that Procter's data conform to the Donnan relation,

$$x^2 = y(y+z),$$

or

$$y+z = x^2/y.$$

Considering the experimental difficulties, the agreement between the last two columns (calculated and observed values of $y+z$) is very satisfactory.

Loeb (97), also, has shown that HCl is distributed unequally between a gelatin gel and the surrounding solution. One gram samples of powdered isoelectric gelatin were placed in solutions of acid of various strengths (volume of each solution = 350 c.c.). The solutions were left for 24 hours at 25°, the relative volume of the swollen particles then measured and the acid solution (external) drained off the gelatin. The latter was now melted by warming to 45°, and the pH values of the melted jelly and of the external solution determined. The jelly was allowed to solidify in a glass cylinder provided with two side-tubes, by means of which connection could be made on the one hand with the external acid and on the other with a saturated solution of KCl. Each of these solutions was in turn connected with a saturated calomel electrode leading to an electrometer. Measurements could thus be made of the electromotive force of the cell:

Cal. (satd.), KCl (satd.), External HCl, Gel, KCl (satd.), Cal. (satd.).

Loeb's results are given in Table XXII. The "calculated" potential difference is the value obtained by application of the formula $E = 0{\cdot}058$ [pH of gel − pH of external solution] (see p. 59). Loeb remarks that, for reasons which he was unable to ascertain, the accuracy of the direct measurement of the potential was

not so great in the case of a gel as when the gelatin was in solution confined within a collodion membrane. However, the agreement between the last two columns of Table XXII is sufficient to show that the systems were in a state of equilibrium. It will be seen that the concentration of the hydrogen ion outside the gel is always greater than inside, which accords with the existence of a Donnan equilibrium, of the nature suggested by Procter.

TABLE XXII.—COMPARISON OF OBSERVED AND "CALCULATED" MEMBRANE POTENTIALS

C.c. 1 M. HCl in 100 c.c. initial solution	Relative volume of gel	pH of melted gelatin	pH external solution	P.D. observed (millivolts)	P.D. "calculated" (millivolts)
0·5	30	4·58	3·89	40·7	37·5
1	40	4·27	3·45	48·4	39·0
2	62	3·76	3·04	42·5	38·0
4	73	3·26	2·65	36·0	29·5
6	75	2·92	2·44	28·4	22·0
8	73	2·57	2·27	17·7	17·7
10	66	2·41	2·16	14·7	17·7
12	64	2·29	2·07	13·2	18·2
15	54	2·11	1·95	9·5	17·0
20	50	1·96	1·82	8·3	10·7
30	41	1·78	1·65	7·7	8·6
40	37	1·59	1·49	5·9	5·4

Procter and Wilson (98) suggest that the swelling due to acid is the result of the excess osmotic pressure in the gel which arises from the difference (e') in concentration of diffusible ions. Equilibrium is established when the osmotic pull is balanced by the cohesive forces of the gelatin molecules or ions which are linked together to form the framework of the jelly. If this is the mechanism

of the swelling, it is evident that the amount of swelling should be greatest when e' reaches its maximum value. The variation of e' with x can be calculated from Loeb's figures (Table XXII), since

$$x^2=y(y+z), \quad \text{or} \quad z=(x^2-y^2)/y,$$

and therefore

$$e'=2y+z-2x=2y+\frac{x^2-y^2}{y}-2x=(x-y)^2/y.$$

The results of these calculations are set out in Table XXIII.

TABLE XXIII.—VARIATION OF e' WITH CONCENTRATION OF ACID

pH of external solution	3·89	3·45	3·04	2·65	2·44	2·27	2·16	2·07	1·95	1·82	1·65	1·49
$e' \times 10^{-3}$	0·4	1·7	3·1	5·0	4·9	2·7	2·4	2·2	1·5	1·6	2·0	1·7

Plotting e' against the pH of the external solution, it appears that the maximum value of e' occurs when the value of the pH lies between 2·44 and 2·65. The corresponding limits for the pH of the gel will be accordingly 2·92–3·26.

Atkin (99) has deduced the pH values for the maximum value of e' from some data due to Procter and Wilson (98). In this case one gram of gelatin was dissolved in 21 c.c. water and the solution kept at 33°, to avoid setting. Hydrochloric acid was added at intervals and the pH of the solution determined (by means of the hydrogen electrode) after each addition. These experiments gave the concentrations of H ion and Cl ion in the various mixtures. Atkin calculated (from $x^2=y(y+z)$)

the value of the pH of the external solution which would be in equilibrium with the acidified gelatin if it were in the form of a gel, and hence was able to obtain values for e'. The graph constructed by Atkin shows a maximum for e' when the pH of the theoretical external solution is 2·3–2·4, which corresponds to a pH in the gelatin of about 3·0.

Table XXIV is a collection of data from a number of sources, and gives observed pH values for maximum *swelling*. It will be seen that these values are in good agreement with those for the maximum of e' obtained above.

TABLE XXIV.—VALUES OF pH AT MAXIMUM SWELLING

Observed by:	Temp. °C.	Acid	pH external solution	pH gel
Procter (96) * . .	18	HCl	2·3–2·4	
Procter (100) * . .	..	HCOOH	2·3–2·5	
Jordan-Lloyd (101) .	20	HCl	2·5–2·6	
Jordan-Lloyd (102) .	0	HCl	2·6	
Jordan-Lloyd and Pleass (103)	18	HCl	2·6	
Loeb (97) . .	20	HCl	2·44	2·92
Northrop and Kunitz (104) . . .	..	HCl	..	3·2
Loeb and Kunitz (41).	..	Various	..	3·0–3·2

Additional confirmation of the theory is afforded by the data of Procter and Burton (105), which show that the increase in volume (V) of a given amount of gelatin is directly proportional to the value of e'. The relation between these quantities is given by $e' = CV$, where C is a constant the value of which depends upon the temperature and other factors.

* See Atkin (99).

Wilson and Wilson (44) * have developed the theory of Procter and Wilson in the following manner. In the equation $e'=CV$, let V represent the increase in volume, in cubic centimetres, of 1 milli-equivalent of gelatin. Then

$$[G]+[GH^+]=\frac{1}{(V+a)},$$

or

$$[G]=\frac{1}{(V+a)}-z,$$

where a is the initial free space within the original dry jelly. Assuming complete ionisation of the gelatin salt, and that the combination of gelatin with hydrogen ion takes place according to the equation

$$[G][H^+]=K[GH^+],$$

or

$$[G]=\frac{Kz}{y};$$

we have

$$\frac{Kz}{y}=\frac{1}{(V+a)}-z,$$

or

$$z=\frac{y}{(V+a)(K+y)} \quad . \quad . \quad (32)$$

Also

$$z=e'-2y+2x$$
$$=e'+2\sqrt{e'y}.$$

Since

$$e'=CV,$$

it follows that

$$z=CV+2\sqrt{CVy},$$

and hence (from equation 32) that

$$(V+a)(K+y)(CV+2\sqrt{CVy})-y=0.$$

* See also Wilson (6), (106).

Now a can be regarded as negligible in comparison with the values of V within the significant swelling range. The above equation therefore reduces to

$$V(K+y)(CV+2\sqrt{CVy})-y=0.$$

Procter and Wilson (*loc. cit.*) found that 768 grams of gelatin combine with a limiting value of 1 mol of HCl and that the combination resembles that of HCl with a weak monoacid base. Adopting the figure 768 as the value of the equivalent weight of gelatin, and employing Procter and Wilson's (*loc. cit.*) values of $K=0{\cdot}00015$ and $C=0{\cdot}0003$,* Wilson (106) has calculated the variation in the adsorption of water by 1 gram of gelatin over the range of acid concentration covered by Procter's (*loc. cit.*) experiments. It will be seen from Fig. 5 that the agreement between the calculated and observed values is quite satisfactory.

If the swelling of gelatin in acids is of the osmotic nature postulated by Procter and Wilson, it follows that the effect of a strong dibasic acid will be considerably less than that produced by a monobasic acid, if comparison is made at the same pH (see p. 74). Fig. 6 represents some of the data of Loeb and Kunitz (41).† In the case of these acids we may say that, except for acetic acid, the degree of swelling depends upon the valency of the anion and not upon the nature of the acid. Since the abnormal behaviour of acetic acid does not occur in either membrane potentials or osmotic pressure, where the effects are due to *isolated* gelatin units, it would appear that the excessive swelling is due to diminution of the cohesion of the gel as the result of the high

* Procter and Burton (*loc. cit.*) give 0·00021 for 18° (the temperature of Procter's experiments). This refers to 1 gram of gelatin, whereas the value 0·0003 refers to 0·768 gram (1 milli-equivalent).

† *Cf.* Ghosh (107), Pleass (108).

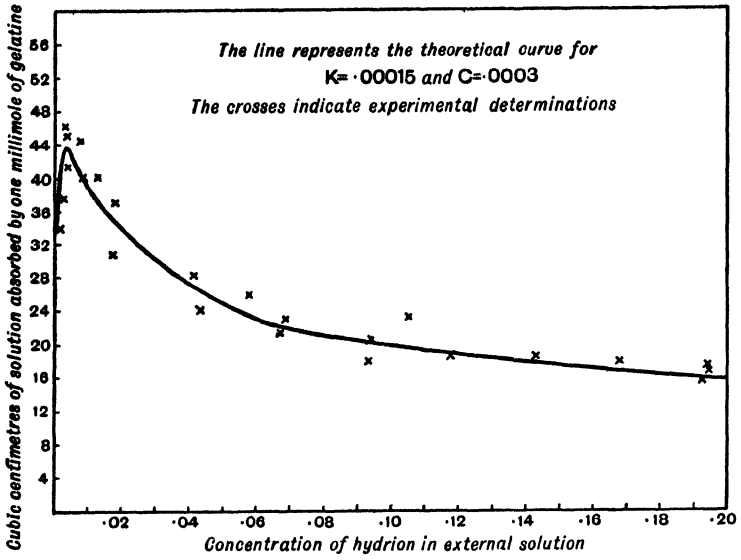


FIG. 5.—Observed and calculated values for the degree of swelling of gelatin in hydrochloric acid solution

(From Wilson, *Journal of the American Leather Chemists' Association*, 1918)

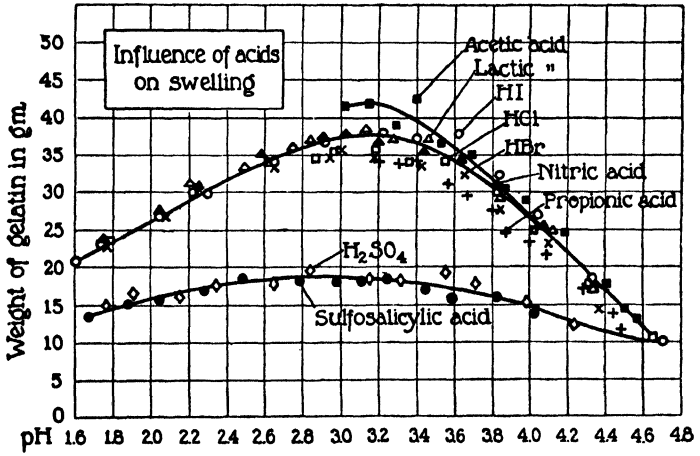


FIG. 6.—Influence of acids on swelling of gelatin

(From Loeb and Kunitz, *Journal of General Physiology*, 1922–1923)

concentration of acetic acid required to bring the pH to 3·2. The effects of certain weak dibasic and tribasic acids (*e.g.* succinic and phosphoric) may be reconciled with the valency rule, if allowance be made for their mode of ionisation (Loeb and Kunitz, *loc. cit.*). On the other hand, Kuhn (109) and Ostwald, Kuhn, and Böhme (110) have shown that in many cases the volume of maximum swelling has no connection with the basicity of the acid. In these instances it would again appear that the cohesive forces of the gel are affected by the acid.

The effects of neutral salts on the swelling of gelatin in regions removed from the isoelectric point are in every way analogous to those produced on the osmotic pressure of gelatin solutions. As Loeb (37) and Loeb and Kunitz (52) have demonstrated, the swelling is repressed, the equilibrium being affected by the salt anion in acid solutions, and its cation in alkaline, and the degree of repression depending upon the valency of the active ion.

According to the theory of Procter and Wilson, the degree of swelling of purified gelatin should depend only on the final pH of the solution in which it is placed, and hence should be independent of the volume of the solution. If, however, the gelatin contains salt impurities, the final concentration of these will depend upon the amount of acid added, and so the degree of swelling will be determined not only by the final pH of the supernatant liquid but also by the volume of acid used. These conclusions have been confirmed by the experiments of Northrop and Kunitz (104).

Table XXII shows that the pH of maximum swelling is independent of the temperature. This is to be expected, since the ionic equilibrium between gel and external solution will not vary appreciably with temperature. On the other hand, according to Procter and Burton (105)

and Jordan-Lloyd (102), the higher the temperature, the greater is the amount of swelling, owing presumably to decreased cohesion of the particles in the framework of the gel.

A word may be said regarding the mechanism which produces the Donnan effect in gels of gelatin. Wilson (6) suggests that the ionised gelatin forms part of the gel framework and hence is non-diffusible. In contrast with this view Jordan-Lloyd (32), (101), (103) * considers that the gelatin ions are present in the liquid phase of the gel and are prevented from diffusing into the outer solution by a framework formed of isoelectric gelatin which therefore acts as a membrane. As far as the osmotic theory of swelling is concerned the only difference between these alternatives is that the gelatin ions in the second case will exert osmotic pressure. The osmotic pressure due to the protein, however, will be small compared with that produced by the difference in concentration of the diffusible ions.

It should be emphasised that the theory of swelling developed by Procter, Wilson, and Loeb cannot be applied indiscriminately. For example, the swelling of dry isoelectric gelatin and of dilute gels of isoelectric gelatin in water and salt solutions must be explained along other lines (Northrop and Kunitz (111) †).

The characteristics of the swelling of gelatin in acid and alkaline ‡ solutions may be taken as typical for proteins in general. Other proteins which have received attention are fibrin (Tolman and Stearn (113), Tolman and Bracewell (114)); casein (Loeb (37)); collagen

* See also Northrop and Kunitz (111).

† See also McBain (43).

‡ See Loeb (37), Kunitz (57), and Pleass (112) for studies on alkaline systems.

(Atkin (115), Porter (116)); and wool (Elöd and Silva (117)).

Tolman and co-workers have proposed an alternative theory of swelling, based on the assumption of electrical repulsions between the different parts of the gel framework. The origin of the electrical field is attributed to the formation of ionic double layers by adsorption of ions. Bennett (118) appears to have suggested a somewhat similar view, which Procter (119) considers untenable since the double layer will be neutral as a whole. In any case Tolman's theory appears to be of a more hypothetical nature than that proposed by Procter and Wilson, and offers no better explanation of the experimental facts. Moreover, the endosmotic experiments of Ghosh (107) indicate that the electric charge on the gelatin particles continues to increase, as the hydrogen ion concentration is increased beyond the value corresponding to maximum swelling, whereas on Tolman's theory the charge should decrease. The theory of Pauli (120), that the swelling is due to the ions of the protein being more heavily hydrated than the neutral molecules, is also not in accord with the observations of Ghosh.

Cellulose.—Procter and Wilson suggested that their theory of swelling would apply to systems other than that of gelatin-acid. Recently Neale (121), (122) has shown that, by adopting their point of view, it is possible to account in an approximately quantitative fashion for the swelling of cellulose in solutions of sodium hydroxide.

The cellulose is regarded as behaving as a very weak monobasic acid, the anions (A^-) and undissociated molecules (HA) of which are joined to form a network possessing mechanical rigidity. Thus the cellulose forms a salt with part of the absorbed alkali. For a given con-

centration (x) of external alkali the equilibrium may therefore be represented by

Cellulose gel (1)		External solution (2)	
	[HA]		
z	A^-		
y	OH^-	OH^-	x
$y+z$	Na^+	Na^+	x
	$[H^+]_1$		

The osmotic pressure (P) tending to drive the water from (2) to (1), and so causing the cellulose to swell, cannot be calculated with precision, but may be considered as very roughly proportional to the difference in total concentration of the diffusible ions, *i.e.*

$$P=P'=RT(2y+z-2x) \quad . \quad . \quad (33)$$

Neale obtains a curve for the variation of P with x, by assuming:

(1) * That the equilibrium is a Donnan equilibrium, *i.e.*

$$y(y+z)=x^2 \quad . \quad . \quad . \quad (34)$$

(2) That each hexose unit contains one acid hydrogen atom, *i.e.* $A=C_6H_9O_5$.

(3) That the ionisation constant (k_a) of the cellulose acid

$$=2{\cdot}0\times10^{-14},\dagger \textit{ i.e. } [H^+]_1 \,.\, z/[HA] = k_a = 2{\cdot}0\times10^{-14} \quad (35)$$

(4) That the cellulose is prevented from swelling when the alkali diffuses into the gel. Thus

$$[HA]+z=\text{constant}=C \quad . \quad . \quad (36)$$

In pure water the particular cellulose used (cellophane) took up about 181 grams of water (at 25°) per one gram

* In place of the usual assumption that the uncombined sodium is at the same concentration (relative to water) in both phases.

† See later. NaA and NaOH are assumed to be completely ionised.

molecule (formula weight in grams) and hence, neglecting the relatively very small concentration of cellulose ions, we have

$$C=[\mathrm{HA}]=5{\cdot}5$$

(since 5·5 moles of cellulose take up 1000 grams of water, and concentrations are expressed in terms of molality).

Substituting for z in equations (33) and (34), by means of (35) and (36), and employing the relation

$$[\mathrm{H}^+]_1 \cdot y = k_w = \text{ionic product of water} = 10^{-14},$$

we arrive at the expressions

$$y\left(\frac{C\beta y}{1+\beta y}+y\right)=x^2,$$

and

$$P=\left(\frac{C\beta y}{1+\beta y}+2y-2x\right)RT,$$

where

$$\beta = k_a/k_w = 2{\cdot}0.$$

The values of x and P obtained by assigning various values of y in these expressions give a theoretical curve for the variation of osmotic pressure with concentration of external alkali when swelling is prevented. Actually the cellulose distends until P is balanced by the cohesive forces, so that the concentration of the cellulose decreases and P does not attain the theoretical value. Neale points out that the assumption that C=constant, greatly simplifies the treatment, since the relation between pressure and swelling is probably quite complex. The value of P calculated as above is therefore to be regarded as a qualitative index of the tendency of the water to enter the gel phase. If the theoretical P–x curve be compared with the experimental absorption curve obtained by Neale for cellophane * (Fig. 7), it will be seen that the

* Washed and dried samples were kept in solutions of alkali at 25° C. ±0·1° C. for two days, rapidly blotted with filter paper and weighed. The

theory depicts with fair accuracy the variation in the effect of the alkali as its concentration is altered.

According to theory, maximum swelling should occur in about 2·7 M alkali, whereas the peak of the experimental curve lies between 3·1 and 3·3 M. However, the employment of a more exact expression for P would have

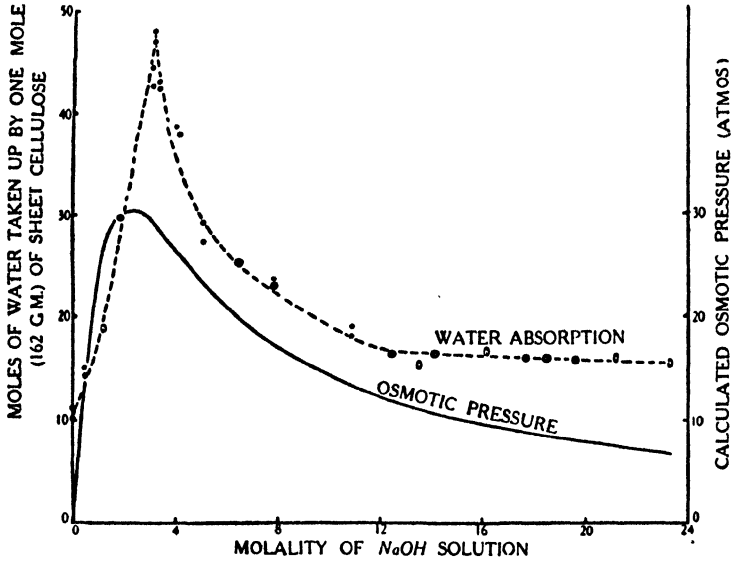


FIG. 7.—Absorption of water by sheets of regenerated cellulose, at 25° C. (From Neale, *Shirley Institute Memoirs*, 1929)

the effect of displacing the theoretical maximum towards the right. With regard to the fact that the swelling curve rises much more steeply to its maximum than does the pressure curve, Neale remarks that this represents the usual relationship between strain and stress in solid materials, the strain increasing more rapidly than the stress when the yield point has been reached. At high

total absorption of alkali (see later) was determined by titrating the swollen sheets with hydrochloric acid. Finally the cellophane was washed free from sodium chloride, dried, and reweighed.

concentrations of alkali the swelling curve no longer falls in accord with the pressure curve. It is possible that another type of action enters here.

Neale (122) has made a separate study of the swelling of cellophane in relatively dilute solutions of alkali, and finds that the swelling is proportional to P over the range 0·1–0·35 molal and then increases more rapidly, as already discussed.

The calculation (on the basis of the theory discussed above) of the total absorption of alkali from the observed absorption of water is a rather more precise process than the calculation of swelling from the concentration of external alkali. Neale (121) has compared the results of such calculations with the values of total alkali absorption found by experiment. His theoretical treatment is as follows, concentrations being expressed as mole ratios (gram molecules per gram molecule of water = molality $\times 18/1000$). For electrical neutrality

$$[Na^+]_1 - [OH^-]_1 = [A^-] \quad . \quad . \quad (37)$$

If W = number of moles of water per mole (gram formula weight) of cellulose, then

$$[HA] + [A^-] = 1/W \quad . \quad . \quad (38)$$

For the ionisation of cellulose and water we have

$$[A^-]/[HA] = \beta f_{OH}[OH^-]_1 \times 55{\cdot}5 * \quad . \quad (39)$$

* $\beta = k_a/k_w$ and f_{OH} = activity coefficient of OH^-. The factor 55·5 is introduced to allow for the conversion of molalities to mole ratios. It would be more correct to put

$$[A^-]/[HA] = \frac{\beta f_{OH}[OH^-]_1}{a_{H_2O}} \times 55{\cdot}5.$$

However, the assumption that the partial pressure of the water-vapour is that of pure water, which is involved in the use of (29), causes little error, for the reason adduced for the approximation of f_{OH} discussed later.

The relations (37), (38), and (39) enable us to eliminate $[Na^+]_1$ from the Donnan equation

$$[Na^+]_1[OH^-]_1=[Na^+]_2{}^2,*$$

and so obtain

$$[OH^-]_1{}^3+\left(\frac{1}{55\cdot5\beta f_{OH}}+\frac{1}{W}\right)[OH^-]_1{}^2 - [Na^+]_2{}^2[OH^-]_1-\frac{[Na^+]_2{}^2}{55\cdot5\beta f_{OH}}=0 \quad . \quad (40)$$

Since water is formed in the reaction postulated between the cellulose and the alkali, W is somewhat greater than the water absorption determined as previously described, and is actually defined by equation (37), the Donnan equation and the stoichiometric relation:

Total swollen weight (per mole cellulose)

$$=18+162[HA]+161[A^-]+23[Na^+]_1+17[OH^-]_1W.$$

Assuming $\beta=2$, and the values for f_{OH} given in Table XXV, we may calculate from (40) the value of $[OH^-]_1$ for any value of $[Na^+]_2$ (external alkali). We know also, from (38) and (39), that the fraction of cellulose present as ionised salt is $\frac{55\cdot5\beta f_{OH}[OH^-]_1}{1+55\cdot5\beta f_{OH}[OH^-]_1}$, and hence can obtain $[Na^+]_1$ in terms of $[OH^-]_1$. Employing the values of the latter derived as above we are enabled to arrive at theoretical values for the total absorption of alkali ($[Na^+]_1$).

Neale writes (*ref.* 121, p. 97): "The published data based on measurements of electromotive force hardly extend beyond 3-molal solution, but the vapour pressure

* The exact equation is, of course, $(a_{Na})_1\times(a_{OH})_1=(a_{Na})_2\times(a_{OH})_2$. However, since the total ionic strengths in the two phases are of the same order, we may assume that the activity coefficient of the alkali is approximately the same in both.

TABLE XXV.—OBSERVED AND CALCULATED ABSORPTION OF SODIUM HYDROXIDE BY CELLULOSE

Experimental			Calculated		
Solution Mole ratio NaOH *i.e.* $[Na^+]_2 = [OH^-]_2$	Gel (per 162 grms. cellulose)		Gel (per 162 grms. cellulose)		Assumed value of f_{OH}
	Total swollen weight (grms.)	Moles of Na^+ *i.e.* $[Na^+]_1 W$	Moles of Na^+ *i.e.* $[Na^+]_1 W$	Mole ratio OH^- *i.e.* $[OH^-]_1$	
0·0	343	0·0	0·0	6×10^{-10}	
0·00868	432	0·265	0·286	0·00388	0·70
0·0219	530	0·672	0·495	0·0126	0·70
0·0354	751	1·412	1·455	0·0260	0·75
0·0556	1067	2·96	2·91	0·0474	0·84
0·0571	1148	3·21	3·20	0·0493	0·85
0·0615	1058	3·08	3·14	0·0528	0·86
0·0720	982	3·15	3·28	0·0619	0·91
0·0928	790	2·99	3·15	0·0782	1·0
0·1179	752	3·44	3·53	0·101	1·0
0·1420	734	3·84	3·92	0·124	1·0
0·1965	663	4·12	4·27	0·173	1·0
0·224	630	4·30	4·37	0·198	1·0
0·243	622	4·56	4·51	0·216	1·0
0·255	646	4·75	4·84	0·227	1·0
0·292	681	5·46	5·58	0·265	1·0
0·319	675	5·62	5·79	0·291	1·0
0·333	689	5·90	6·06	0·305	1·0
0·353	696	6·19	6·33	0·325	1·0
0·382	727	6·86	6·96	0·354	1·0
0·419	734	7·28	7·34	0·390	1·0

data, whilst not sufficient to justify the assignment of activity coefficients, indicate that f_{OH} rises considerably above unity. It is fortunate, however, that f_{OH} only

occurs in Equation (xii),* and that its increase merely increases the fraction of cellulose salt. Since beyond 5 M this fraction always exceeds 0·9, and rises steadily, further increase of f_{OH} is without serious effect, and for the more concentrated solutions f_{OH} has been taken as unity for the purpose of the present calculations."

It will be seen from Table XXV that the agreement between the calculated and observed values of the total alkali absorption is quite good, except at the two lowest concentrations. To explain this divergence, Neale suggests that owing to the relatively small amount of swelling in dilute solutions, a small fraction of the acid groups is inaccessible to the alkali. With increase in the swelling, the whole of the cellulose acid becomes available, and the law of molecular proportions is consequently more closely obeyed.

The choice, in the above calculations, of $2{\cdot}0 \times 10^{-14}$ as the value of the ionisation constant of cellulose was made on rather arbitrary grounds. In his second paper (122) Neale describes the evaluation of k_a by means of measurements in dilute solutions of alkali (below 0·5 molal). Such measurements are more suitable for the purpose than those made at technically important concentrations, since the properties of concentrated solutions of alkali are not yet well defined, and also because the fraction of combined alkali decreases as the concentration increases. From determinations of the absorptions of water and of the distribution of alkali, the constant was calculated from the formula,

$$k_a = \frac{k_w[A^-]}{f_{OH}[OH^-][HA]},\dagger$$

* *I.e.* equation (39) on p. 119 of this book.

† The experiments were carried out at 25° C. with cellophane. k_w was taken as $1{\cdot}005 \times 10^{-14}$. Values for f_{OH} were derived from given

by means of the relations (35), (37), and (38) given above. The values of k_a so obtained showed satisfactory constancy. Their mean value was $1{\cdot}84 \times 10^{-14}$, and it will be seen that the use of this figure in place of $2{\cdot}0 \times 10^{-14}$ would not materially affect the quantitative relations of the preceding discussions.

Swelling of Animal Tissues

In many respects the swelling in aqueous solutions of fresh or dried animal tissues resembles that of gelatin.

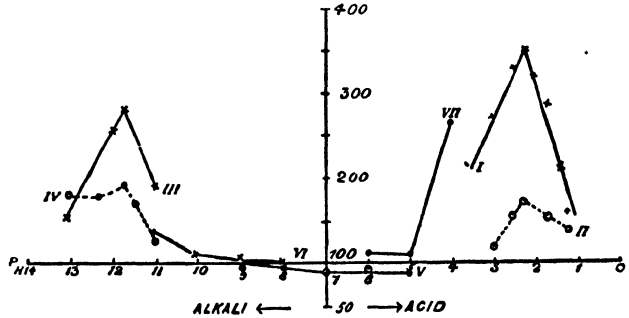


Fig. 8.—Swelling of the sterno-cutaneous muscle of the frog

I. In hydrochloric acid.
II. In Ringer's solution and hydrochloric acid.
III. In sodium hydroxide.
IV. In Ringer's solution and sodium hydroxide.
V. In M/15 phosphate buffer solution.
VI. In M/10 borate buffer solution.
VII. In M/5 acetate buffer solution.

Ordinates represent weight of 100 parts of moist, freshly excised muscle.
(From Jordan-Lloyd, *Proceedings of the Royal Society*, B, 1916)

Owing, however, to their characteristic structure the swelling in the case of tissues is much less pronounced. Each cell acts as an isolated gel, and, owing to the proximity of the neighbouring cells, has only a restricted space in which to expand. The influence of the composition of the solution on the swelling of the sterno-cutaneous muscle of the frog is illustrated by the curves in Fig. 8, which reproduces the experimental data of a

data, on the assumption that $f_{OH}=f_{Na}$. This probably introduces less error than putting $f_{OH}=1$.

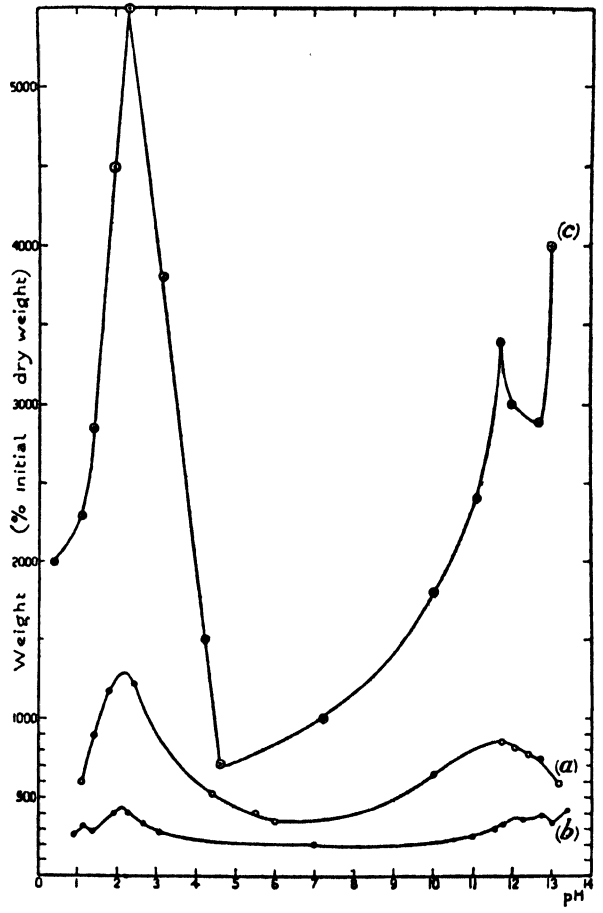


FIG. 9.—Influence of structure on the degree of swelling
Curve *a*, fresh goatskin.
,, *b*, dried goatskin.
,, *c*, leaf gelatin.
(From Kaye and Jordan-Lloyd, *Proceedings of the Royal Society*, B, 1924)

research by Jordan-Lloyd (123). Fig. 9 shows the influence of pH on the swelling of goatskin as observed by Kaye and Jordan-Lloyd (124). By comparison with the curve for leaf gelatin, included in Fig. 9, it will be realised how similar are the various systems. It should

be noted that the pH values for the maximum swelling of the goatskin are close to those for gelatin, but that the degree of swelling is very much less than that of the protein.

The repressive action of salts in the zones of maximum acid and alkaline influence is shown by the data of Kaye and Jordan-Lloyd (124) for dry goatskin (Fig. 10). It will be noted that other effects become evident at the iso-

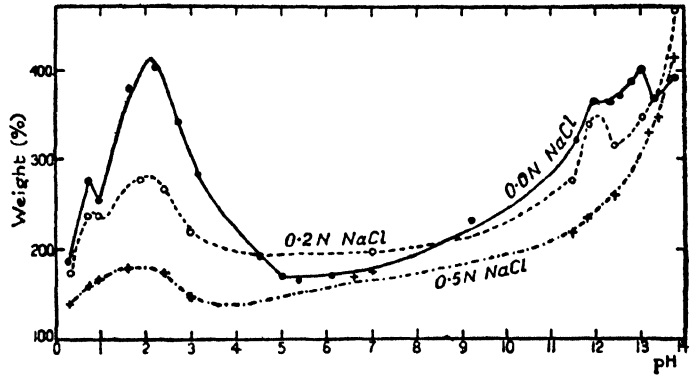


FIG. 10.—Influence of sodium chloride on the swelling of dry goatskin (From Kaye and Jordan-Lloyd, *Proceedings of the Royal Society*, B, 1924)

electric point and in strongly alkaline solutions, where the Donnan effect is negligible.*

THE MANUFACTURE OF LEATHER †

A proper understanding of the factors involved in the swelling of animal skins is of course essential for the scientific control of the various operations employed in the manufacture of leather. It was the need in this connection for some reliable theory of swelling that prompted Procter to investigate the behaviour of gelatin.

* *Cf.* Page and Page (125).
† Procter (126), Wilson (127), Gerngross (128).

Some examples are given here of the application of the theory of Procter, Wilson, and Loeb to the interpretation of the swelling changes undergone by hides in the course of their conversion into leather.

As a preliminary treatment the hides are steeped in milk of lime for the purpose of loosening the epidermis and facilitating the removal of the hair. At the same time the swelling which takes place brings the fibrils of collagen present in the middle part of the skin into a condition suitable for tanning. Lime forms the basis of the bath, but sodium sulphide may be added to hasten the decomposition of the keratinous substances. In addition to its "sharpening" action, however, the sulphide increases the swelling. This is in accord with theory, since the sulphide by hydrolysis and interaction with the lime gives rise to sodium hydroxide, and the theory predicts that under comparable conditions swelling with a monoacid base will be greater than with a diacid base (see p. 111).

Excessive swelling is particularly undesirable in the preparation of the softer and finer grades of leather. It follows that in the case of these, sulphide must be introduced into the liming bath in such a form that sodium hydroxide is either not present at all or else only in small amount. This may be accomplished by the use of calcium hydrosulphide, formed by passing hydrogen sulphide into milk of lime, or by the interaction of arsenic sulphide with hot slaked lime (see Atkin (115)); or by the addition of a mixture of equivalent proportions of calcium chloride and sodium sulphide (Procter (*loc. cit.*)). These substitutes have been found to give the desired effects in practice, but of course differ in cost and convenience.

If a salted hide is not washed prior to liming, the salt may promote swelling in the liming bath. Atkin (*loc.*

cit.) has pointed out that this increased swelling is due to the formation of sodium collagenate according to the equilibrium,

$$Ca(\text{Collagenate})_2 + 2Na^+ \rightleftharpoons 2\text{Na Collagenate} + Ca^{++}.$$

The procedure subsequent to the removal of epidermis and hair depends upon the quality of leather in view. In any case the excess of lime in the hide must be removed, and for normal tannage the pH of the "pelt" or unhaired hide brought to the acid side of the isoelectric point of collagen (pH 4·7). For the production of sole and heavy leathers simple treatment with acid is often sufficient, and the problem here is to avoid overswelling as the lime is replaced by acid, while maintaining the "plumpness" reached in the liming bath. According to theory this is a matter of adjusting the pH, which may be effected most conveniently by employing (as is commonly done in actual practice) acids such as boric and acetic, since in these cases the pH does not vary rapidly with concentration.

For the production of the finer types of leather, the process of liming is followed by that of "bating." In modern practice the washed skins are immersed in a bath consisting chiefly of ammonium chloride and pancreatin. Depletion and deliming take place, and a further portion of non-collagenous material is removed. The depletion may be attributed, at least in part, to the decrease in alkalinity of the skin and to the repressive action of the salts.

In the process of "pickling," by which the "pelt" is rendered suitable for preservation in a soft state, the material is first allowed to swell in acid and is then depleted by the addition of salt. Here again, in all probability, the changes which occur involve the operation

of osmotic forces in the manner postulated by Procter, Wilson, and Loeb.

Swelling of Cotton Hairs

Neale (122) has applied equation (40) to calculate the total absorption of sodium hydroxide from the absorp-

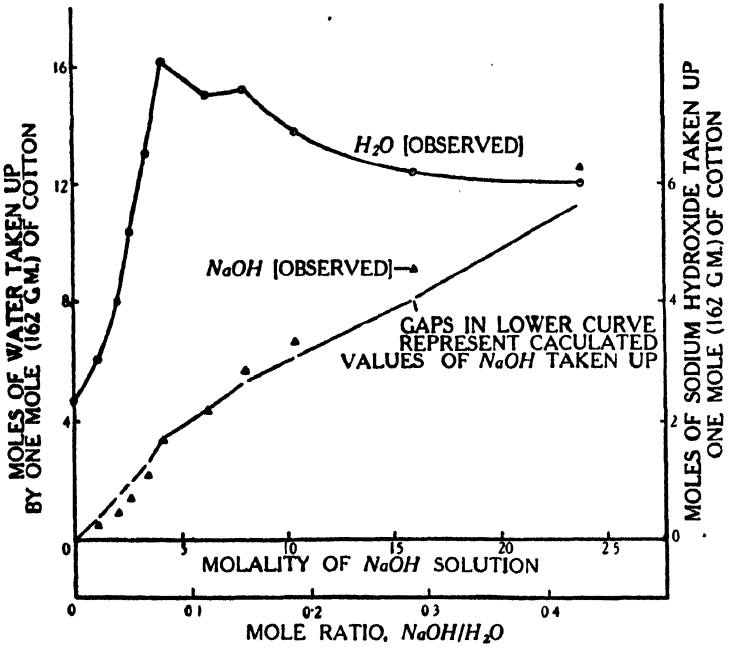


FIG. 11.—Absorption of sodium hydroxide by cotton
(From Neale, *Shirley Institute Memoirs*, 1929)

tion of water by cotton hairs, as determined by Coward and Spencer (129). The results are compared with the observed values of alkali absorption in Fig. 11, which shows that the discrepancies are not greater than might be expected in view of the assumptions made and the rather large experimental errors. It is evident that the inflexion in the alkali absorption curve is the consequence

of the maximum in the swelling curve, and does not represent discontinuous compound formation. As in the case of cellophane, the observed alkali absorption at low concentrations is less than the predicted. The explanation advanced in the case of cellophane may be applied here also, and is supported by the fact that the divergence disappears in the region of maximum swelling, and by the observation of Vieweg (130) that the more swollen mercerised cotton takes up more alkali from dilute solutions than does untreated cotton.

Despite the complications due to the presence of a structure, the swelling curve of Coward and Spencer, Fig. 11, is in qualitative agreement with the theory, the maximum occurring in solutions of moderate concentration.

Dyeing

Elöd and Silva (117) have recently discussed the application of the Donnan theory to another process of technical interest, namely, the dyeing of animal fibres by acid dyes. The main features of the discussion are as follows.

Since wool, natural silk, etc., are protein in nature, fibres of these materials behave in the same manner as gelatin on immersion in a solution of, for example, hydrochloric acid. That is to say, at equilibrium the concentration of hydrogen ion in the swollen fibre is less than in the external solution. If now an acid dyestuff (anion = dye) is added to the solution, the dye will be distributed unequally in the opposite sense. This follows from the Donnan relation,

$$\frac{[H^+]_s}{[H^+]_f} = \frac{[A^-]_f}{[A^-]_s},$$

where A^- is the anion of the dye, and s and f represent

the solution and the fibre respectively. The presence of the acid thus facilitates the penetration of the dye into the fibrous material.

As previous considerations (p. 58) show, when the acidity of the solution is continuously increased, the value of $[H^+]_s/[H^+]_f$ passes through a maximum on the acid side of the isoelectric point. Hence for a given initial concentration of dye there exists an optimum pH at which the proportion of dye taken up by the fibre is greatest.

The addition of a neutral salt, such as sodium sulphate, to the dye solution decreases the inequality in the distribution of all the ions (p. 11). Hence, in the presence of salt less dye diffuses into the solution held by the fibre and the rate of fixation of the dye is correspondingly reduced. Since the dyestuff itself is a salt, increase in its concentration also leads to more equal distribution. In this case, however, while the fraction of dye in the fibre decreases, the absolute amount increases.

Elöd and Silva apply equation (6) (p. 11) to an approximate calculation of the influence of the concentration of the dyestuff or neutral salt upon the distribution ratio. They put c_1=the concentration of protein chloride, thereby neglecting the small amount of free acid present in the fibre; c_2=the concentration of hydrochloric acid in the external liquid; and c_3=the initial concentration of uni-univalent dye or other salt. The values of c_1 and c_2 were obtained from experiments on hide material. Table XXVI contains the results of the calculations. In addition to illustrating the preceding remarks, the figures show that the maximum value of the distribution ratio occurs at a lower pH value when the concentration of dye is increased.

TABLE XXVI.—VARIATION OF $1+\frac{c_1}{c_2+c_3}$ $\left(i.e.\ \frac{[A^-]_f}{[A^-]_a}\right)$ WITH c_3

$-\log c_2$	$c_3=0{\cdot}002$	$c_3=9{\cdot}016$
0·93	2·6	2·4
1·06	3·3	2·9
1·19	3·9	3·4
1·32	4·3	3·6
1·89	8·5	4·9
2·67	14·4	4·1
3·11	14·0	3·2
3·46	12·5	2·7
3·79	7·0	1·8
4·18	4·6	1·4
4·78	3·0	1·3
4·86	1·8	1·1
5·12	1·4	1·05

CHAPTER IV

PHYSICO-CHEMICAL APPLICATIONS

In this chapter consideration is given to certain applications which, at the present stage, are primarily of physico-chemical interest. That they may become of wider significance is readily seen.

Determination of Cataphoretic Velocity

If we imagine a layer of hydrochloric acid solution superposed upon a layer of protein solution containing hydrochloric acid at the same pH, the system will not be in equilibrium. This arises out of the fact that the protein has such a low rate of diffusion that the boundary between the solutions is equivalent to a membrane permeable for hydrogen and chloride ions but not for the protein units. If we represent the system by

$$\begin{array}{ll} (2) & H^+ \quad Cl^- \\ \hline (1) & H^+ \quad Cl^- \quad P^{+'} \end{array}$$

the initial condition is

$$[Cl^-]_2=[H^+]_2=[H^+]_1<[Cl^-]_1.$$

That is to say,

$$[H^+]_1[Cl^-]_1>[H^+]_2[Cl^-]_2.$$

Since the equilibrium is of the Donnan type the final state will be

$$[H^+]_1[Cl^-]_1=[H^+]_2[Cl^-]_2.$$

Acid must therefore diffuse from (2) to (1) and the pH of (2) become less than that of (1).

If now the boundary is brought under the influence of an electric field which causes the protein to migrate into the pure acid solution, the protein will not move at a uniform speed, since its charge, and hence mobility, depends upon the pH of the solution, which, as we have seen, will not be constant. In this way unreliable values may be obtained for the cataphoretic velocities of proteins, when determined by the U-tube method.*

This source of error has been discussed at length by Tiselius (131), who points out that it may be reduced to negligible proportions by adding a neutral salt in sufficient quantity, since, according to the theory of Donnan, this produces equal distribution of all the ions present.

Since, in cataphoretic determinations, it is desirable to keep the electrolyte concentration as low as possible in order to minimise the heating effect of the current, it is of importance to ascertain the minimum concentration of salt which suffices to repress the Donnan effect to the desired extent. Tiselius shows how the necessary calculation is to be made in the case of a uni-univalent electrolyte.

Consider, for example, the addition of sodium nitrate to the system. In this case the equilibrium may be represented by

$$\begin{matrix} z & P^+ \\ y & \left\{\begin{matrix} H^+ \\ Na^- \end{matrix}\right. \end{matrix} \left.\begin{matrix} \\ \end{matrix}\right\} \begin{matrix} Cl^- \\ \left.\begin{matrix} \\ NO_3^- \end{matrix}\right\} z+y \end{matrix} \quad \Big| \quad x \left\{\begin{matrix} H^+ & Cl^- \\ Na^+ & NO_3^- \end{matrix}\right\} x,$$

where z is the charge on the colloid in equivalents per unit of volume.

* It will be realised that similar considerations apply to other colloids. See McBain (43) and Weiser (132) for criticism of the data of Varga, Wintgen, and Zsigmondy for sols of stannic oxide. See also Wintgen (133).

Then

$$y=\frac{-z+\sqrt{z^2+4x^2}}{2}, \text{ (see p. 72)}$$

or

$$y+z=\frac{z+\sqrt{z^2+4x^2}}{2}.$$

Hence

$$\frac{y+z}{x}=\frac{z+\sqrt{z^2+4x^2}}{2x},$$

which is equivalent to the equation obtained by Tiselius.

Tiselius applies this relation to the case of a 0·4 per cent. egg-albumin solution with a pH value differing by $\pm 0{\cdot}5$ unit from that at the isoelectric point. Judging from the amount of acid bound by the protein, z has the value of 40×10^{-5} equivalents per litre under these conditions. In Table XXVII will be found data calculated

TABLE XXVII.—DEPRESSION OF DONNAN EFFECT BY NEUTRAL SALTS

x	$\frac{y+z}{x}$	$\log(y+z)-\log x$
10^{-4}	4·2	0·62
10^{-3}	1·2	0·08
10^{-2}	1·02	0·009

from this value of z, on the assumption that z remains constant. The third column gives values for the calculated difference in pH between the two solutions, and the table shows that a salt concentration of about 0·01 M is sufficient to maintain equality between the pH values on opposite sides of the boundary.

Determination of Molecular Weight by Centrifuge *

Within the last few years Thé Svedberg has devised methods of determining the molecular weight of proteins by the application of centrifugal force (Ultracentrifuge). Provided the latter is not too great, a state of "sedimentation equilibrium" is finally attained, owing to the balance of centrifugal and diffusive forces. Leaving electrical forces out of account, the expression for the molecular weight is

$$\mathbf{M}=\frac{2RT\ln(c_2/c_1)}{(1-V\rho)\omega^2(x_2^2-x_1^2)},$$

where V is the partial specific volume of the protein, ρ the density of the solvent, ω the angular velocity, and c_2 and c_1 are the concentrations of the protein at the respective distances x_2 and x_1 from the centre of rotation.

As will be gathered from the above expression, a concentration gradient is produced in the column of protein by the centrifugal action. If the protein is not at the isoelectric point and is present in salt-free solution, this protein concentration gradient will give rise to a Donnan potential gradient which exerts an effect in opposition to that of the centrifugal field. It will be seen that the potential involved corresponds to that produced at a membrane separating solutions of a non-diffusible electrolyte at different concentrations.†

Tiselius (135) has deduced the effect of the electrical forces upon the equilibrium state in the following manner:—

Let $$\pi=-\int_0^x(1-V\rho)\omega^2\,.\,x\,.\,dx.$$

* See Svedberg (134).

† For example, a copper ferrocyanide membrane separating solutions of potassium ferrocyanide.

Then $d\pi = -\frac{RT}{\mathbf{M}_0} d \ln a$, where a is the activity of the protein salt (PA_n).

But $$d \ln a = d \ln (a_{n+} \times a_-^n),$$

where a_{n+} is the activity of the protein cation and a_- that of the anion.

Hence $$\mathbf{M}_0 d\pi = -RTd \ln a_{n+} - RTnd \ln a_-.$$

Assuming complete dissociation and replacing activities by concentrations, we therefore have (when protein salt alone is present)

$$\mathbf{M}_0 d\pi = -RT(n+1) d \ln c,$$

since c = concentration of protein ion and $c_- = nc$.

Integration gives

$$\mathbf{M}_0 = \frac{2RT(n+1) \ln (c_2/c_1)}{(1 - \bar{V}\rho)\omega^2(x_2^2 - x_1^2)}.$$

Hence the observed value for the molecular weight is $n+1$ times smaller than the true value, if electrical forces are neglected.

This is, however, the maximum effect. The presence of free acid or of salts represses the potential gradient, and in sufficient concentration practically eliminates it altogether. Table XXVIII (Nichols (136)) gives an idea of the error which may be introduced by neglect of the Donnan potential. In this case the protein was subjected to a centrifugal force 2700 times gravity. The calculated values of c_2/c_1 were derived by taking $\mathbf{M}$ as equal to 68,500, which appears to be quite definitely the value of the molecular weight of hæmoglobin under the conditions of the experiment.

TABLE XXVIII.—DONNAN EQUILIBRIUM IN SEDIMENTATION

x_2 (cm.)	x_1 (cm.)	log c_2/c_1		Ratio a/b
		Observed (a)	Calculated (b)	
4·68	4·63	0·0289	0·0422	0·685
4·63	4·58	0·0262	0·0418	0·627
4·58	4·53	0·0257	0·0413	0·622
4·53	4·48	0·0265	0·0409	0·648
4·48	4·43	0·0267	0·0404	0·661
4·43	4·38	0·0261	0·0400	0·653
4·38	4·33	0·0266	0·0396	0·672
4·33	4·28	0·0267	0·0391	0·683
4·28	4·23	0·0263	0·0386	0·682
			Average . .	0·659

It will be seen that the ultracentrifuge may be employed to obtain some indication of the mean valence of a protein, since centrifuging a sample of the acid or alkaline solution free from salts, and a second containing sufficient salt to reduce the Donnan potential to negligible proportions, permits of the approximate evaluation of $n+1$.

VISCOSITY OF PROTEIN SOLUTIONS

Broadly speaking, two types of viscous behaviour are met with in dilute solutions of proteins. In the case of albumin, for example, the rise in viscosity with increasing concentration is slow, as in a solution of a crystalloid. Gelatin, on the other hand, shows very different behaviour under the same conditions, since a small increase in concentration produces a marked change in viscosity.

If we accept the view that increase in viscosity is to be ascribed solely to increase in the relative volume of

the solute, it is evident that the amount of water associated with the protein must be much greater in gelatin than in albumin solutions. Following up this line of reasoning, Loeb (34), (35) has suggested that in solution gelatin exists partly as aggregates which swell to form submicroscopic gels, whereas albumin dissolves

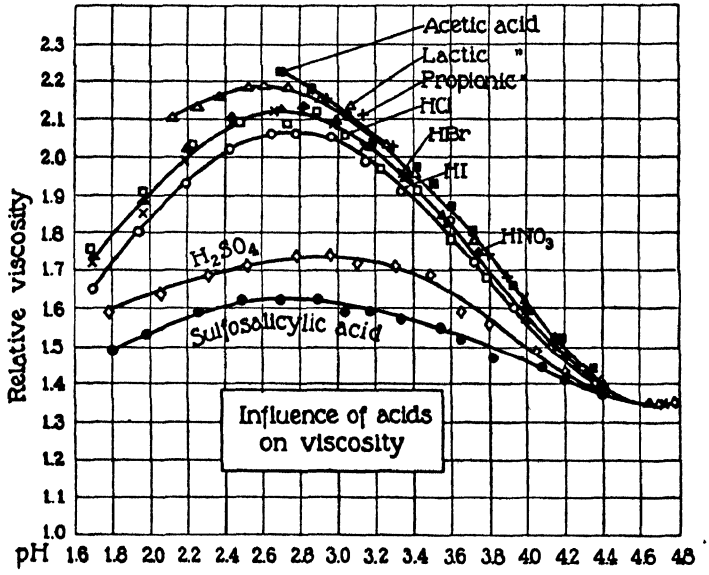


FIG. 12.—Influence of acids on the relative viscosity of gelatin solutions (From Loeb and Kunitz, *Journal of General Physiology*, 1922–1923)

in the state of isolated molecules. On this hypothesis, the viscosity of gelatin should vary with the hydrogen-ion concentration in the same manner as does swelling. The viscosity coefficient is conceived to be a function of the volume of the swollen aggregates, and this is in turn a function of the distribution of the diffusible ions between the aggregates and the surrounding solution. Viscosity-pH curves (Fig. 12) obtained by Loeb and Kunitz (41) actually do bear a strong resemblance to

those for swelling and osmotic pressure. In contrast with this, the viscosity of an albumin solution undergoes relatively little change with change in the hydrogen-ion concentration (Loeb, *loc. cit.*).

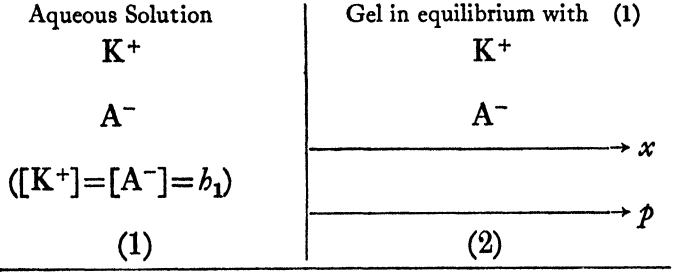
In a recent paper Kunitz (137) has calculated the "hydration" or water content of the gelatin micelle from the formula *

$$\eta = \frac{1 + 0{\cdot}5\phi}{(1-\phi)^4},$$

where η = the relative viscosity of the solution, and ϕ = the volume occupied by the solute, expressed as c.c. of solute per c.c. of solution. The "hydration" value so obtained permits of the further calculation of the osmotic pressure difference between the inside and the outside of the micelle, due to the unequal distribution of the diffusible ions. The osmotic pressure arrived at in this way agrees well with that derived directly from the activities of the ions.

Distribution Gradient within a Gel

In a recent paper Donnan (139) draws attention to the influence of variable micellar concentration upon the distribution of ions in the system gel-aqueous solution. The equilibrium relations may be deduced by a consideration of the following scheme:—



* Kunitz (138).

To simplify matters it will be assumed that the uni-univalent electrolyte KA is completely ionised, and that p, the number of micelles per unit volume, alters in some definite continuous fashion with the distance from the gel-solution boundary, *i.e.* that $p=\phi(x)$.* Also the gel micelles are considered to combine only with the ion K^+, the combination being reversible. Taking some definite point x in the gel, let p be the concentration of micelles, p_i the number of bound K^+ per unit volume, and h the average concentration of free K^+. The average concentration of A^- must therefore be $h+p_i$. It should be pointed out that the "active" concentration of A^- will be $h+p_i(1-\alpha)$, where α represents the influence of the electric charge of the micelle. In the case of a uni-univalent electrolyte in sufficiently low concentration we may put $\alpha=0$ without serious error.

The conditions at two neighbouring points x and $x+\delta x$ in the gel may therefore be represented by—

	x	$x+\delta x$
Micelle	p	$p+\delta p$
Bound K^+	p_i	$p_i+\delta p_i$
Free K^+	h	$h+\delta h$
Free A^-	$h+p_i$	$(h+\delta h)+(p_i+\delta p_i)$

Now

$$h(h+p_i)=(h+\delta h)(h+\delta h+p_i+\delta p_i).\dagger$$

* It is also assumed that p does not vary in a direction parallel to the gel-solution boundary.

† Strictly speaking this equation is only exact if the symbols represent activities.

Hence
$$\frac{dh}{dx} = -\frac{h}{2h+p_i},$$

and
$$\frac{d(A^-)}{dx} = \frac{d(h+p_i)}{dx} = \frac{h+p_i}{2h+p_i} \cdot \frac{dp_i}{dx} \quad . \quad (41)$$

The same result is of course reached by differentiating the equation $h(h+p_i)=$constant $(=h_1^2)$ with respect to x.

In order to use equation (41) we must make some assumption as to the relation between p and p_i, since we are given the form of the function $p=\phi(x)$, but not that of $p_i=\omega(x)$. If we regard the combination of K^+ with micelle as an adsorption which obeys the simplest type of Langmuir equation, we have
$$p_i = \frac{ah}{1+bh},$$

where a and b are constants. Also $p_i=pN\Theta$, where $N=$number of active points per micelle, and $\Theta=$number of active points occupied by K^+ ions. For large values of h we have
$$p_i = \frac{a}{b} = Np, \quad \text{or} \quad a = bNp.$$

Hence
$$p_i = \frac{bhN}{1+bh}p = \frac{ch}{1+bh}p,$$

where c and b are constants. In this case we have the distribution equation,
$$h\left(h+\frac{chp}{Hbh}\right) = \text{constant } (=h_1^2).$$

Differentiating with respect to x we obtain
$$\frac{dh}{dx} = -\frac{ch}{2(1+bh)+cp+\dfrac{cp}{1+bh}} \cdot \frac{dp}{dx}.$$

The corresponding general equation for $[A^-]$ is easily

deduced, since $b[A^-]$=constant, and therefore

$$\frac{d[A^-]}{dx} = -\frac{[A^-]}{b} \cdot \frac{db}{dx}.$$

From this it follows that

$$\frac{d[A^-]}{dx} = \frac{c[A^-]}{2(1+bh)+cp+\frac{cp}{1+bh}} \cdot \frac{dp}{dx}.$$

These equations lead to the conclusion that the distribution, in the gel, of the ions K^+ and A^-, will take the form of two concentration gradients, of opposite directions. If $\frac{dp}{dx}>0$, then $\frac{db}{dx}>0$ and $\frac{d[A^-]}{dx}>0$.

As one result of these equilibrium gradients there will be a potential gradient *within* the gel. Let e_0 and e be the positive potentials for $x=x_0$ and $x=x$ respectively. Then

$$e-e_0=\frac{RT}{\mathbf{F}} \log \frac{[K^+]_0}{[K^+]_x}=\frac{RT}{\mathbf{F}} \log \frac{[A^-]_x}{[A^-]_0}.$$

The variable concentration of the diffusible electrolyte must also give rise to a corresponding variation in the extent of swelling of the gel.

Itmight appear that the recent experiments of Bigwood (140) afford examples of the type of system here described. This worker reports the presence of a permanent concentration gradient when, under suitable conditions, dilute aqueous sodium hydroxide diffuses into a gelatin gel. Halpern (141) has carried out similar experiments with hydrochloric acid for the purpose of finding conditions under which Donnan's theoretical expressions might be tested. In these cases, however, the diffusion resulted in uniform distribution of the electrolyte, and the conclusion was reached that all the conditions imposed upon Bigwood's experiments would lead one to

expect an extreme case of slow diffusion which might be mistaken for a stationary state. Light scattering experiments * also failed to detect any permanent gradient for protein micelles in gels of initially uniform composition.

Halpern also tested the effect of a pre-formed gelatin concentration gradient (obtained by layering equal amounts of gelatin sols upon each other) upon the diffusion of HCl. After 4½ months the hydrogen-ion concentration was still not evenly distributed, as would have been the case with a uniform gel under the same conditions. Analysis of different sections of the gel showed that the hydrogen-ion concentration decreased gradually from the top section down, while the chloride concentration increased in the same direction, the gel being least concentrated at the top (see Table XXIX). Donnan's conclusions were thus verified in a general way.

TABLE XXIX.—DISTRIBUTION GRADIENT IN A GEL

Concentration gelatin (grams per cent.)	Chloride per gram of gelatin (milligrams)
1·89	0·408
1·87	0·399
2·07	0·445
2·23	0·493
2·54	0·494
4·28	0·673
6·49	0·780

ELECTROKINETIC POTENTIALS

The movement of solid particles through a liquid or of liquid along a solid wall (so-called electrokinetic pheno-

* Krishnamurti (see Halpern, *loc. cit.*).

mena), under the influence of an electric field, is usually attributed to the existence of a potential difference at the surface of the solid. The suggestion that this potential might be comparable with a membrane potential was first put forward by J. A. Wilson (6), (142), whose treatment is as follows:—

Consider a solid surface in contact with a solution of an electrolyte MN, a proportion of whose cations are bound to the solid by some force or other. Since the corresponding anions are held by electrostatic attraction in the neighbourhood of the surface, the concentration of N^- near the surface will be greater than that of the free M^+. At some distance from the surface the concentrations will of course be equal. The equilibrium is evidently of the Donnan type, since it is set up as the result of a constraint imposed upon certain of the ions which prevents their free diffusion. Thus if we represent the film of solution wetting the solid by (1), and the bulk of the solution by (2), the distribution should conform to the relation

$$[M^+]_1[N^-]_1=[M^+]_2[N^-]_2,$$

or

$$\frac{[M^+]_1}{[M^+]_2}=\frac{[N^-]_2}{[N^-]_1}=\lambda.$$

This unequal distribution of ions will produce a difference in potential between the surface film of liquid and the surrounding solution, the value of which is given by

$$E=\frac{RT}{\mathbf{F}}\ln\frac{1}{\lambda}.$$

It is obvious that increasing the electrolyte content of the solution will decrease the value of E. Wilson has advanced an explanation of the coagulation of colloidal particles from this point of view.

BIBLIOGRAPHY

1. Ostwald, *Z. physikal. Chem.*, 1890, **6**, 71.
2. Donnan and Harris, *J. Chem. Soc.*, 1911, **99**, 1554.
3. Donnan, *London Physiological Society*, December 1910.
4. Donnan, *Z. Electrochem.*, 1911, **17**, 572.
5. Gibbs, "The Scientific Papers of J. Willard Gibbs," 1906, **1**, 83; "The Collected Works of J. Willard Gibbs," 1928, **1**, 83. (New York and London.)
6. Wilson, "Colloidal Behaviour," Bogue, 1924, **1**, 1. (New York and London.)
7. Donnan and Garner, *J. Chem. Soc.*, 1919, **115**, 1314.
8. Donnan, *Chem. Rev.*, 1924, **1**, 73.
9. Donnan, *Solvay Conseil Intern. de Chim.*, April 1928.
10. Hückel, *Kolloid-Z. (Zsig. Festschrift)*, 1925, **36**, 204.
11. Kameyama, *Phil. Mag.*, 1925, **50**, 849.
12. Adair, *Science*, 1923, **58**, 13.
13. Donnan, *J. Franklin Instit.*, 1925, **199**, 471.
14. Hitchcock, *J. Gen. Physiol.*, 1926, **9**, 97.
15. Donnan and Allmand, *J. Chem. Soc.*, 1914, **105**, 1941.
16. Lewis and Randall, "Thermodynamics," 1923, **1**. (New York and London.)
17. Debye, *Physikal Z.*, 1924, **25**, 97.
18. Kameyama, *Phil. Mag.*, 1927, **3**, 235.
19. Donnan and Green, *Proc. Roy. Soc.*, 1914, **A, 90**, 450.
20. Azuma and Kameyama, *Phil. Mag.*, 1925, **50**, 1264.
21. Bayliss, *Proc. Roy. Soc.*, 1909, **B, 81**, 269.
22. Biltz and Vegesack, *Z. physikal. Chem.*, 1910, **73**, 481.
23. Biltz, *Z. physikal. Chem.*, 1913, **83**, 625.
24. Robinson and Mills, *Proc. Roy. Soc.*, 1931, **A, 131**, 576 and 596.
25. Bjerrum, *Z. physikal. Chem.*, 1924, **110**, 656.
26. Rinde, *Phil. Mag.*, 1926 (**S 7**) **1**, 32.
27. Ganguly and Krishnamurti, *Trans. Faraday Soc.*, 1928, **24**, 401.
28. Hammarsten, E. and H., *Arkiv. Kemi. Min. Geol.*, 1923, **8**, 1; Hammarsten, H., *Biochem. Z.*, 1924, **147**, 481.
29. Ghosh, *J. Chem. Soc.*, 1929, pp. 2290 and 2298.

30. Ganguly, *J. Physical Chem.*, 1927, **31**, 407.
31. Lewis, *Chem. Rev.*, 1931, **8**, 81.
32. Jordan-Lloyd, "Chemistry of the Proteins," 1926. (London.)
33. Hitchcock, *J. Gen. Physiol.*, 1921–1922, **4**, 597; *J. Gen. Physiol.*, 1922–1923, **5**, 383.
34. Loeb, "Proteins and the Theory of Colloidal Behaviour," 1922. (New York and London.)
35. Loeb, "Colloidal Behaviour," Bogue, 1924, **1**. (New York and London.)
36. Hitchcock, *Physiol. Rev.*, 1924, **4**, 505; *Ergeb. der Physiol.*, 1924, **23** (1), 274.
37. Loeb, *J. Gen. Physiol.*, 1921, **3**, 85, 547, 667, 691.
38. Hill, *Proc. Roy. Soc.*, 1923, **A**, **102**, 705; *J. Gen. Physiol.*, 1923–1924, **6**, 91.
39. Hitchcock, *J. Gen. Physiol.*, 1922–1923, **5**, 661; 1923–1924, **6**, 93.
40. Hitchcock, *J. Gen. Physiol.*, 1921–1922, **4**, 733.
41. Loeb and Kunitz, *J. Gen. Physiol.*, 1922–1923, **5**, 665.
42. Rideal, "Surface Chemistry," 1930, p. 430. (Cambridge.)
43. McBain, "Colloid Symposium Monograph," 1926, **4**, 7. (New York.)
44. Wilson and W. H. Wilson, *J. Amer. Chem. Soc.*, 1918, **40**, 886.
45. Hitchcock, *J. Amer. Chem. Soc.*, 1926, **48**, 2870.
46. Sporing, *J. Chem. Soc.*, 1924, **125**, 2316.
47. Wright, *Biochem. J.*, 1929, **23**, 352.
48. Pauli, "Colloid Chemistry of the Proteins," 1922, p. 102. (London.)
49. Northrop and Kunitz, *J. Gen. Physiol.*, 1925–1926, **9**, 351; 1928, **11**, 481.
50. Simms, *J. Gen. Physiol.*, 1927–1928, **11**, 613.
51. Thimann, *J. Gen. Physiol.*, 1930, **14**, 215.
52. Loeb and Kunitz, *J. Gen. Physiol.*, 1922–1923, **5**, 692.
53. Loeb, *Science*, 1922, **56**, 731.
54. Loeb, *J. Amer. Chem. Soc.*, 1922, **44**, 1930.
55. Hitchcock, *J. Gen. Physiol.*, 1922, **5**, 35.
56. Loeb, *J. Gen. Physiol.*, 1920–1921, **3**, 691.
57. Kunitz, *J. Gen. Physiol.*, 1923–1924, **6**, 547.
58. Lillie, *Amer. J. Physiol.*, 1907–1908, **20**, 127.
59. Sørensen, *Compt. rend. Trav. Lab. Carlsberg*, 1915–1917, **12**; *Zeit. physiol. Chem.*, 1919, **106**, 43.

60. Adair, *Proc. Roy. Soc.*, 1928, **A**, **120**, 573; *J. Amer. Chem. Soc.*, 1929, **51**, 692.
61. Adair and Callow, *J. Gen. Physiol.*, 1929–1930, **13**, 565.
62. Harwood, *J. Chem. Soc.*, 1923, p. 2254.
63. Hammarsten, E., "Veroff. der Knut und Alice Wallenberg-Stiftung," 1924, **3**; *Biochem. Z.*, 1924, **144**, 383.
64. Murray, *J. Gen. Physiol.*, 1923–1924, **6**, 759.
65. Henderson, "Blood: A Study in General Physiology," 1928. (Yale University Press.)
66. Warburg, *Biochem. J.*, 1922, **16**, 153.
67. Van Slyke, "Factors affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body," 1926 (Philadelphia and London); "Colloidal Behaviour," Bogue, 1924, **1**, 98 (New York and London).
68. Van Slyke, Wu, and McLean, *J. Biol. Chem.*, 1923, **56**, 765.
69. Van Slyke, Hastings, Heidelberger, and Neill, *J. Biol. Chem.*, 1922, **54**, 481.
70. Van Slyke, Hastings, Murray, and Sendroy, *J. Biol. Chem.*, 1925, **65**, 701.
71. Bock, Dill, Hurxthal, Lawrence, Coolidge, Dailey, and Henderson, *J. Biol. Chem.*, 1927, **73**, 749.
72. Hastings, Sendroy, jun., McIntosh, and Van Slyke, *J. Biol. Chem.*, 1928, **79**, 193.
73. Adair, *J. Biol. Chem.*, 1925, **63**, 517.
74. Hastings and Van Dyke, *J. Biol. Chem.*, 1928, **78**, 35; (*Proc. Amer. Soc. Biol. Chem.*), "Colloid Symposium Monograph," 1928, **6**, 153. (New York.)
75. Hastings, Harkins, and Leiter, "Colloid Symposium Monograph," 1928, **6**, 153. (New York.)
76. Taylor, *Proc. Roy. Soc.*, 1924, **B**, **96**, 383.
77. Henriques, *Ergeb. der Physiol.*, 1929, **28**, 625.
78. Loeb, R. F., *J. Gen. Physiol.*, 1925–1928, **8**, 451.
79. Loeb, R. F., and Nichols, *J. Biol. Chem.*, 1927, **72**, 687.
80. Loeb, R. F., and Nichols, *J. Biol. Chem.*, 1927, **74**, 645.
81. Nitschke, *Biochem. Z.*, 1925, **165**, 229.
82. Nitschke and Freyschmidt, *Biochem. Z.*, 1926, **174**, 287.
83. Duke-Elder, *Brit. J. of Ophthalmology*, Monograph Supp. III, 1927.
84. Duke-Elder, *The Lancet*, 4th January 1930, 4.
85. Duke-Elder, *Biochem. J.*, 1927, **21**, 66.
86. Duke-Elder, *Brit. J. of Ophthalmology*, Monograph Supp. IV, 1930.

87. Duke-Elder, *J. Physiol.*, 1927, **62**, 315.
88. Colle, Duke-Elder, P. M., and Duke-Elder, *J. Physiol.*, 1931, **71**, 1.
89. Lehmann and Meesmann, *Pflüger's Archiv.*, 1924, **205**, 210.
90. Hastings, Salvesen, Sendroy, jun., and Van Slyke, *J. Gen. Physiol.*, 1925–1928, **8**, 701.
91. Loeb, R. F., Atchley, and Palmer, *J. Gen. Physiol.*, 1921–1922, **4**, 591.
92. Gollwitzer-Meier, *Z. ges. exp. Med.*, 1925, **46**, 15.
93. Michaud, "Conférence à la Soc. helvet. des Sciences nat.," 4th October 1924; *Virchow's Archiv.*, 1925, **254**, 710; *Schweiz. med. Woch.*, 12th June 1926.
94. Mestrezat, "La Médicine," September 1925; *Compt. rend. Soc. Biol.*, 1921, **85**, 55.
95. Northrop, *J. Gen. Physiol.*, 1924, **6**, 337; 1925, **7**, 603.
96. Procter, *J. Chem. Soc.*, 1914, **105**, 313.
97. Loeb, *J. Gen. Physiol.*, 1921–1922, **4**, 351.
98. Procter and Wilson, *J. Chem. Soc.*, 1916, **109**, 307.
99. Atkin, *J. Soc. Leather Trades' Chem. Assoc.*, 1920, 248.
100. Procter, *Kolloidchem. Beih.*, 1911, **2**, 243; *J. Amer. Leather Chem. Assoc.*, 1911, **6**, 270.
101. Jordan-Lloyd, *Biochem. J.*, 1920, **14**, 147 and 584.
102. Jordan-Lloyd, *Biochem. J.*, 1930, **24**, 1460.
103. Jordan-Lloyd and Pleass, *Biochem. J.*, 1927, **21**, 1352.
104. Northrop and Kunitz, *J. Gen. Physiol.*, 1928–1929, **12**, 537.
105. Procter and Burton, *J.S.C.I.*, 1916, **35**, 404.
106. Wilson, *J. Amer. Leather Chem. Assoc.*, 1918, **13**, 177.
107. Ghosh, *J. Chem. Soc.*, 1928, p. 711.
108. Pleass, *Biochem. J.*, 1929, **23**, 358.
109. Kuhn, *Kolloidchem. Beih.*, 1921, **14**, 147.
110. Ostwald, Kuhn, and Böhme, *Kolloidchem. Beih.*, 1925, **20**, 412.
111. Northrop and Kunitz, *J. Physical Chem.*, 1931, **35**, 162.
112. Pleass, *Biochem. J.*, 1930, **24**, 1472.
113. Tolman and Stearn, *J. Amer. Chem. Soc.*, 1918, **40**, 264.
114. Tolman and Bracewell, *J. Amer. Chem. Soc.*, 1919, **41**, 1503.
115. Atkin, *J. Ind. Eng. Chem.*, 1922, **14**, 412.
116. Porter, *J. Soc. Leather Trades' Chem. Assoc.*, 1921, **5**, 259.
117. Elöd and Silva, *Z. physikal. Chem.*, 1928, **137**, 142.
118. Bennett, *J. Soc. Leather Trades' Chem. Assoc.*, 1918, **2**, 40.
119. Procter, *J. Soc. Leather Trades' Chem. Assoc.*, 1918, **2**, 73.

120. Pauli, *Biochem. Z.*, 1909, **18**, 368.
121. Neale, *Shirley Inst. Mem.*, 1929, **8**, 87; *J. Textile Inst.*, 1929, **20**, 373T.
122. Neale, *Shirley Inst. Mem.*, 1930, **9**, 21; *J. Textile Inst.*, 1930, **21**, 225T.
123. Jordan-Lloyd, *Proc. Roy. Soc.*, 1916, **B**, **89**, 277.
124. Kaye and Jordan-Lloyd, *Proc. Roy. Soc.*, 1924, **B**, **96**, 293.
125. Page and Page, *J. Ind. Eng. Chem.*, 1927, **19**, 1264.
126. Procter, "Colloidal Behaviour," Bogue, 1924, **2**, 700 (New York and London); "Principles of Leather Manufacture," Procter, 1922 (London); "B.A. Report on Colloid Chemistry," 1917, p. 5.
127. Wilson, "Chemistry of Leather Manufacture," Wilson, 1923, p. 128 (New York); *J. Ind. Eng. Chem.*, 1922, **14**, 834; *J. Ind. Eng. Chem.*, 1929, **21**, 180.
128. Gerngross, *Z. angew. Chem.*, 1928, **41**, 221 and 254.
129. Coward and Spencer, *J. Textile Inst.*, 1923, **14**, 32T.
130. Vieweg, "Summary of the Literature on Mercerisation," Clibbens, *Shirley Inst. Mem.*, 1923, **2**, 157; *J. Textile Inst.*, 1923, **14**, 217T.
131. Tiselius, *Nova Acta Soc. Sci.*, 1930, Ser. IV, **7**, 34.
132. Weiser, *Z. physikal. Chem.*, 1931, **35**, 1.
133. Wintgen, *Kolloid-Z.*, 1926, **40**, 141.
134. Svedberg, *Kolloid-Z.* (*Zsig. Festschrift*), 1925, **36**, 63–64; *Trans. Faraday Soc.*, 1930, **26**, 744–746.
135. Tiselius, *Z. physikal. Chem.*, 1926, **124**, 449.
136. Nichols, *J. Amer. Chem. Soc.*, 1930, **52**, 5176; "Colloid Symposium Monograph," 1928, **6**, 287 (New York).
137. Kunitz, *J. Gen. Physiol.*, 1927, **10**, 811.
138. Kunitz, *J. Gen. Physiol.*, 1925–1926, **9**, 715.
139. Donnan, *Kolloid-Z.*, 1930, **51**, 24.
140. Bigwood, *Trans. Faraday Soc.*, 1930, **26**, 704.
141. Halpern, *J. Gen. Physiol.*, 1931, **14**, 575.
142. Wilson, *J. Amer. Chem. Soc.*, 1916, **38**, 1982.
143. Hastings and Sendroy, *J. Biol. Chem.*, 1925, **65**, 445.

INDEX OF AUTHORS

Adair, G. S., 27, 76.
Allmand, A. J., 39, 40, 41, 45.
Atchley, D. W., 100.
Atkin, W. R., 108, 115, 126.
Azuma, R., 45 *et seq.*

Bayliss, W. M. (Sir), 49.
Bennett, H. G., 115.
Bigwood, E. J., 142.
Biltz, W., 49.
Bjerrum, N., 52, 54.
Bock, A. V., 147.
Böhme, E., 113.
Bracewell, R. S., 114.
Burton, D., 109, 113.

Callow, E. H., 76.
Colle, J., 148.
Coolidge, T. C., 147.
Coward, H. F., 128.

Dailey, M. E., 147.
Debye, P., 41.
Dill, D. B., 147.
Donnan, F. G., 1, 2, 3, 5, 11, 14, 19, 21, 23, 27, 31, 39 *et seq.*, 48, 49, 58, 61, 78, 139.
Duke-Elder, P. M., 148.
Duke-Elder, W. S., 95 *et seq.*

Elöd, E., 115, 129 *et seq.*

Freyschmidt, H. J., 94.

Ganguly, P. B., 52, 54.
Garner, W. E., 5, 41, 42, 78.
Gerngross, O., 125.
Ghosh, B. N., 54, 55, 56, 115.
Gibbs, W. J., 2, 27.
Gollwitzer-Meier, K. (Mme.), 100.
Green, G. M., 43, 44, 48.

Halpern, L., 142.
Hammarsten, E., 77, 145.
Hammarsten, H., 54.
Harkins, H. N., 88.
Harris, A. B., 1, 45, 48, 49.
Harwood, F. C., 76.
Hastings, A. B., 83, 86, 87, 88, 91, 100.
Heidelberger, M., 83.
Henderson, L. J., 81, 86, 87.
Henriques, O. M., 87, 89 *et seq.*
Hill, A. V., 60.
Hitchcock, D. I., 28 *et seq.*, 56, 61, 65, 70, 73 *et seq.*
Hückel, E., 23, 25, 41, 76.
Hurxthal, L. M., 147.

Jordan-Lloyd, D. (Miss), 56, 66, 109, 114, 123, 125.

Kameyama, N., 26, 40, 41, 43 *et seq.*
Kaye, M. (Miss), 124, 125.
Krishnamurti, K., 143.
Krishnamurti, S., 52, 54.
Kuhn, A., 113.
Kunitz, M., 61, 62, 63, 67, 68, 73, 109, 111, 113, 114, 138, 139.

Lawrence, J. S., 147.
Lehmann, G., 99.
Leiter, L., 88.
Lewis, G. N., 40, 46, 92.
Lewis, W. C. McC., 56, 66.
Lillie, R. S., 76.

Loeb, J., 57 *et seq.*, 68, 70 *et seq.*, 80, 106, 108, 109, 111, 114, 126, 128, 138.
Loeb, R. F., 94, 100.

McBain, J. W., 66, 114, 133.
McIntosh, J. F., 87.
McLean, F. C., 81 *et seq.*
Meesmann, A., 99.
Mestrezat, W., 100.
Michaud, L., 100.
Mills, H. A. T., 49 *et seq.*
Murray, C. D., 78, 86, 87.

Neale, S. M., 115 *et seq.*, 128.
Neill, J. M., 83.
Nichols, E. G., 94.
Nichols, J. B., 136.
Nitschke, A., 94.
Northrop, J. H., 67, 101 *et seq.*, 109, 113, 114.

Ostwald, Wi., 1, 4.
Ostwald, Wo., 113.

Page, A. W., 125.
Page, R. O., 125.
Palmer, W. W., 100.
Pauli, W., 67, 115.
Pleass, W. B. (Miss), 114.
Porter, E. C., 115.
Procter, H. R., 72, 80, 103 *et seq.*, 125, 126, 128.

Randall, M., 46, 92.
Rideal, E. K., 66.
Rinde, H., 52, 53, 54, 66.
Robinson, C., 49 *et seq.*

Salvesen, H. A., 100.
Sendroy, J., 86, 87, 91.
Sendroy, J. (jun.), 87, 100.
Silva, E., 115, 129 *et seq.*
Simms, H. S., 68.
Sørensen, S. P. L., 76.
Spencer, L., 128.
Sporing, P. A., 66.
Stearn, A. E., 114.
Svedberg, T., 135.

Taylor, H., 88.
Thimann, K. V., 68.
Tiselius, A., 133, 135.
Tolman, R. C., 114, 115.

Van Dyke, H. B., 88.
Van Slyke, D. D., 81 *et seq.*, 93, 100.
Varga, G., 133.
Vegesack, A. von, 49.
Vieweg, 129.

Warburg, E. J., 81, 91.
Weiser, H. B., 133.
Wilson, J. A., 3, 66, 72, 80, 107 *et seq.*, 126, 128.
Wilson, W. H., 66, 110.
Wintgen, R., 133.
Wright, N. C., 66.
Wu, H., 81 *et seq.*

Zsigmondy, R., 133.

INDEX OF SUBJECTS

Acidosis, 88.
Activities, 23.
Adsorption of ions, 52, 54, 65, 66.
Albumin, 73, 76.
Alkalosis, 88.
Aqueous humour, 94 *et seq.*

Benzopurpurin-4B, 49.
  isomer, 49 *et seq.*
Blood—
  bromide distribution in, 88.
  distribution equilibria in, 80 *et seq.*
  human, 87.
  oxygenation of, 86.
  state of calcium in, 94.
Brillantkongo, 49.

Carrageen, 76, 77.
Casein, 66, 67, 73, 114.
Cataphoresis, 132 *et seq.*
Cellulose, 115 *et seq.*
Centrifuge, 135 *et seq.*
Cerebro-spinal fluid, 100.
Chromic hydroxide, 54.
Collagen, 114, 127.
Congo-red, 1, 3, 45 *et seq.*
Cotton, 128 *et seq.*

Dyeing, 129 *et seq.*
Dyes, 45 *et seq.*

Edestin, 73.
Electrokinetic potentials, 143, 144.
Enzymes, 101 *et seq.*

Ferrocyanides, 39 *et seq.*
Fibrin, 114.

Gelatin—
  combination with neutral salts, 67 *et seq.*
  gels, 103 *et seq.*, 142 *et seq.*
  solutions (see Proteins).
Gels—
  cellulose, 115 *et seq.*
  distribution gradient in, 139 *et seq.*
  gelatin, 103 *et seq.*
  membrane potentials, 107.
  swelling of, 107 *et seq.*
Globulin, 73.

Hæmoglobin—
  combination with salts, 89 *et seq.*
  ionic nature of, 88.
  membrane potentials, 88 *et seq.*
Hydration, 136.

Intraocular fluids, 94 *et seq.*
Ionic strength, 40, 41.

Kinetic theory—
  common diffusible ion, 3.
  common non-diffusible ion, 5.
  no common ion, 8.
  polyvalent ions, 7.
  several diffusible electrolytes, 10, 18.
Kongoreinblau, 49.

Leather—
  bating, 127.
  deliming, 127.
  liming, 126.
  pickling, 127.

MEMBRANES—
amyl alcohol, 78.
collodion, 47.
cupric ferrocyanide, 39.
Membrane hydrolysis, 21, 49.
Membrane potentials—
aqueous humour, 99.
Congo-red, 48.
ferrocyanides, 43.
gels, 107.
hæmoglobin, 88 *et seq.*
proteins, 59 *et seq.*, 67 *et seq.*
stannic acid sols, 55.
sulphur sols, 53.
theory of, 14 *et seq.*, 60, 61.
Molecular weight—
by centrifuge, 135 *et seq.*
by osmotic pressure, 20, 76.
Muscle (frog), 123.

NEUTRALISATION across a membrane, 28 *et seq.*
neutral salts, 36.
non-diffusible acid, 33.
non-diffusible ampholyte, 33, 35.
non-diffusible base, 29, 36.

ŒDEMA fluids, 100, 101.
Osmosis, 25.
Osmotic pressure—
dyes, 50, 51.
inorganic colloids, 53 *et seq.*
molecular weight determinations, 20, 76.
Osmotic pressure (*continued*)—
proteins, 70 *et seq.*
theory, 19 *et seq.*
thymonucleates, 77.

PARACENTESIS, 98, 99.
Polyvalent ions, 7, 13, 17, 42.
Proteins—
combination with neutral salts, 67 *et seq.*, 89 *et seq.*
distribution relations, 56 *et seq.*
membrane potentials, 59 *et seq.*, 69.
osmotic pressure, 70 *et seq.*
viscosity, 137 *et seq.*

SILICATES, 54.
Silicic acid sols, 54.
Skin (goat), 124, 125.
Stannic acid sols, 54 *et seq.*
Sulphur sols, 52 *et seq.*

THERMODYNAMIC theory,
general, 2 *et seq.*
simple, 11 *et seq.*
Theory of solutions, 41, 54.
Thymonucleates, 77.
Tissues (animal), 123 *et seq.*

VISCOSITY, 137 *et seq.*
Vitreous body, 97 *et seq.*

WOOL, 115, 129.